the absence of a specific teaching, and that the efficacy of H_2O_2 with regards to sterilization is concentration dependent. In support of this position, Applicants submitted U.S. Patent 4,643,876 as Exhibit A with the previous response. It was further noted that the irradiation times in Ikada, et al. are either not provided or they are very short and it was respectfully submitted that these time periods are not long enough to allow for sterilization at the stipulated energy levels.

Furthermore, Applicants argued that as the order of the steps and the plasma power are important, there is no assurance that whatever sterilization may inherently occur would be compatible with the coating material and there is no assurance that sterilization would be maintained throughout the subsequent coating steps.

The Examiner's position appears to be that since hydrogen peroxide is safe, common, affordable and readily available, one of ordinary skill in the art would likely choose hydrogen peroxide to use in the method of Ikada, et al, and, at that point, sterilization would have become inherent. Regarding Applicants' arguments regarding insufficient concentrations of hydrogen peroxide, insufficient sterilization times and the order of the process steps, the Examiner maintains that some degree of sterilization would still occur.

In response, sterilization is not a matter of degree. Sterilization either occurs or it does not occur. This is the art accepted definition of sterilization which is discussed in the attached Exhibit A (DISINFECTION, STERILIZATION, AND PRESERVATION (5th edition) Seymour S. Block, ed., Lippincott, Williams & Wilkins, Philadelphia, 2001). The Examiner's attention is directed to page 21, col. 2, para. 2, which states "Sterilization is the act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms". In the next paragraph, the reference states that "sterilization was intended to convey an absolute meaning, not a relative one. A substance cannot be partially sterile". Thus, "some degree of sterilization" does not constitute "sterilization" as the term is defined in the art. As Applicants' claims require a sterilization step, the Examiner's assertions of "partial sterilization" do not teach or suggest the claimed process.

On page 25, col. 2 of Exhibit A, the AAMI definition of "sterile" is provided as

Sterile: The state of being free from all living microorganisms; in practice, usually described as a probability function, such as the probability of a surviving microorganism being one in a million (AAMI, 1995).

Finally, on page 885, col. 1-2, bridging para., the reference states:

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms. This state of sterility is the objective of the sterilization procedure and, viewed in this context, the definition is categorical and absolute; that is, an item is either sterile or it is not.

Thus, the Examiner's statements that "some degree of sterilization still occurs" (Paper No. 5, page 5, para. 1), "nor has Applicant provided any proof that any such a timeframe is insufficient for *any* level of sterilization to occur" (Examiner's emphasis, page 5, para. 2), and "it appears that *some* sporicidal activity or sterilization will occur at any level" (Examiner's emphasis, page 6, para. 1) are meaningless within the art-accepted definition of sterilization because there is no such thing as partial sterilization.

The Examiner also argues that Applicant does not claim any concentration requirement for sterilization (Paper No. 5, page 5, para. 1), that Applicants do not claim a time for sterilization (page 5, para. 2) and that Applicant has not claimed that sterilization lasts any specified amount of time (page 6, para. 1). In response, both independent claims 1 and 16 recite a sterilizing step. Consequently, sterilization is already an element of the claimed method. As discussed above with respect to Exhibit A, sterilization is an absolute term - it either is sterile or it is not. In contrast, in the teaching of Ikada, et al., there is no teaching or suggestion that sterilization occurs.

In view of Applicants' arguments and Exhibit A, reconsideration and withdrawal of this ground of rejection is respectfully requested.

Claims 1-5, 8, and 16-18 are rejected under 35 U.S.C. § 103(a) as unpatentable over Akagi et al. (U.S. Patent No. 4,728,564).

The Examiner notes that Akagi requires a peroxide source in the same manner as required by Ikada and refers to statements made with regards to the Ikada reference as the arguments are similar.

Applicants also refer to comments made with regards to Ikada et al. discussed above which are incorporated herein by reference.

Regarding the Examiner's comment on the peroxide source, while Akagi et al. teach the use of O_2 has to form a low temperature plasma (col. 9, lines 53-66), there is no teaching or suggestion in Akagi, et al. on sterilization and there is reason to believe that sterilization would not necessarily occur under the conditions taught by Akagi as discussed previously and supported by U.S. Patent 4,643,876 discussed in the last Response. Consequently, sterilization is not

inherent to the method of Akagi et al. As discussed above, the concept of partial sterilization is not recognized in the art. Sterilization either occurs or it does not.

There is no teaching in Akagi et al. on sterilization. Additionally, Applicants have presented reasons why sterilization would not occur under the conditions described by Akagi et al with the last response. With this response, Applicants present an art accepted definition of sterilization that does not include partial sterilization. In view of the above, Applicants respectfully submit that Akagi, et al does not teach sterilization and does not teach conditions under which sterilization as defined in the art would occur.

In view of the above, reconsideration and withdrawal of this ground of rejection is respectfully requested.

Claims 1, 3, 5-15, and 19 are rejected under 35 U.S.C. § 103(a) as unpatentable over Hendriks (U.S. Patent No. 5,866,113) in view of Spencer (U.S. Patent No. 5,656,238).

As set forth by the Examiner, Hendriks teaches graft polymerization of medical devices followed by sterilization with ethylene oxide. Spencer teaches that a plasma of hydrogen peroxide can be used for sterilization.

In contrast, Applicants claimed invention provides for a process whereby both the polymerization of the bioactive coating and sterilization are facilitated by hydrogen peroxide gas plasma. This may occur either simultaneously in one step or in two separate steps with polymerization of the bioactive material occurring first followed by the sterilization step. While the polymerization of the bioactive coating may be carried out by any means known in the art, the hydrogen peroxide gas plasma will enhance this process. Furthermore, it was totally unexpected in the art at the time of the claimed invention that hydrogen peroxide gas plasma could be used to sterilize any material containing a bioactive coating because one of ordinary skill in the art would have expected the gas plasma to destroy the bioactive coating as discussed further below.

Thus, Applicants argue that the two references are not combinable, and that, even if they are combinable, that the results obtained by Applicants were unexpected. One of ordinary skill in the art would not substitute ethylene oxide gas of Hendriks, et al. for the hydrogen peroxide gas plasma of Applicants' claimed invention because ethylene oxide is a gas which cannot be used to form radicals to initiate polymerization of the bioactive coating. It is important to note that the hydrogen peroxide gas plasma of Applicants' claimed method potentially serves two

purposes. First, it serves as a sterilizing agent. Second, it serves as a source of free radicals for polymerization of the bioactive coating. One of ordinary skill in the art would not choose ethylene oxide to initiate a polymerization reaction because ethylene oxide is a gas which does not generate free radicals. In support of this position, Applicants present Exhibit B (Jacobs & Lin "Gas-Plasma Sterilization" Chapter 17 in IRRADIATION OF POLYMERS (Clough & Shalaby, eds.), ACS Symposium Series 620, August 21-26, 1994, American Chemical Society, Washington, D.C., 1996).

On page 217, paragraph 2, of Exhibit B, the reference states that "Plasma is defined as a fourth state of matter, energetically distinguishable from solids, liquids, and gases." Thus, plasma is a different species from ethylene oxide which is a gas. Unlike gas plasma, it cannot modify the material surface. In contrast, the hydrogen peroxide gas plasma is capable of forming radicals which are required for the polymerization of the bioactive coating. The Examiner's attention is directed to Exhibit B at page 218, paragraph 1, which teaches that "For that reason, these plasmas have the unique properties of having electrons or other species that have sufficient energy to cause the rupture of molecular bonds while the temperature of atoms and molecules in the plasma are near ambient values." That is, even though the temperature of the plasma is low, there is enough energy to create radicals at the surface of the material being sterilized. For this reason, the plasma can cause polymerization on the surface of the bioactive coating. On page 220 of Exhibit B, the reactions in plasma for which hydrogen peroxide serves as a precursor are set forth in simplified form. Ethylene oxide gas is not capable of creating these radicals. Thus, one of ordinary skill in the art would not expect to substitute ethylene oxide gas for hydrogen peroxide gas plasma because the ethylene oxide is a gas which, unlike hydrogen peroxide gas plasma, is not capable of forming radicals to form the bioactive coating.

Furthermore, it could not have been predicted based upon the knowledge in the art at the time that the claimed invention was made that the free radicals produced by the hydrogen peroxide gas plasma would not destroy or be deleterious to the bioactive coating. Note that gamma radiation produces free radicals in a similar manner to hydrogen peroxide gas plasma. These radicals are capable of surface modification whether they are produced by gas plasma or by gamma radiation (see Exhibit B, page 231). Thus, one of ordinary skill in the art would have expected that both gas plasma and gamma radiation would affect the surface of a material having a bioactive coating in the same manner. However, this is not the case. As can be seen from

Table 2 of the present specification, the hydrogen peroxide gas plasma treatment is the only treatment tested that preserves the bioactive coating. Gamma radiation does not preserve the bioactive coating. This result was unexpected and could not have been predicted from the knowledge in the art at the time of the claimed invention.

In view of Applicants' arguments and Exhibit B as discussed above, Applicants request that the Examiner reconsider and withdraw the above ground of rejection.

CONCLUSION

In view of Applicants' amendments to the claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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EXHIBIT A

Disinfection, Sterilization, and Preservation

Fifth Edition

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do not disinfect people or wounds. We treat them with antiseptics. The idea of cleaning goes hand-in-hand with disinfection, and the distinction may be confused. The legal dictionary *Words and Phrases* tells of a court case in which a company that disinfected a railroad for carrying food left it dirty and was sued. The judge explained that disinfection did not mean that all dirt had to be removed, as long as it did not contain living microorganisms capable of doing harm; thus the case was lost. Whereas the word *disinfectant* was originally and still is used primarily with regard to pathogenic microorganisms in a medical setting, the more general use would cover a range of nonmedical organisms and applications, such as a toilet bowl disinfectant, water cooling-tower disinfectant, or drilling-mud disinfectant. It can be concluded from this analysis that dictionary definitions in this area cannot be counted on for strict, modern meanings. They can be used as a beginning, but one would be better advised to consult professionals actively working in the field for more accurate, complete interpretation of the terms.

A chemical may have different antimicrobial designations depending on its application. For example, Fraser (1987) mentions a number of designations for peracetic acid: "It should be noted the terms used for disinfectants vary between industries, e.g., 'sanitizers' in food and brewing industries, 'terminal disinfectants' in dairies, 'biocides' in municipal water purification, 'ovicides' in agricultural waste stabilization, 'sterilants' in medicinal and pharmaceutical applications." Of course, peracetic acid may be bacteriostatic under certain conditions with short contact time, bactericidal with longer time, and sporicidal or biocidal with even longer time. Each is a proper designation for the conditions of use. On the other hand, some industries may use terminology peculiar to that industry with no regard to properly accepted definitions. It would be desirable to have definitions accepted internationally, but they are not; therefore, there may be different interpretations of the terms as they are used in different countries.

ANTISEPTIC

An *antiseptic* is a substance that prevents or arrests the growth or action of microorganisms, either by inhibiting their activity or by destroying them. The term is used especially for preparations applied topically to living tissue.

The legal definition (Federal Food, Drug and Cosmetic Act, 1938) states: "the representation of a drug in its labeling as an *antiseptic* shall be considered to be a representation that it is a germicide, except in the case of a drug purporting to be, or represented as, an *antiseptic* for inhibitory use as a wet dressing, ointment, dusting powder, or other such use as involves prolonged contact with the body."

Comments

The word *antiseptic*, derived from the Greek "against putrefaction," was used by Pringle in 1750 (see Chapter 1) to record the ability of substances to prevent the spoilage of organic matter such as egg and meat. After Lister's research in the use of antimicrobial agents in surgery, the term acquired a second meaning, that of a substance used to destroy pathogenic microorganisms. There was some difference of opinion in regard to the second meaning—whether an antiseptic merely inhibited the growth of microorganisms, killed them, or both. Patterson examined 165 definitions of *antiseptic* used between 1819 and 1930, of which 20 make no mention of bacteria; 27 state that they arrest but do not destroy, 12 that they destroy, 10 that they inhibit growth but do not indicate how, 66 that they do one or the other, and 30 that antiseptics act to neutralize toxins and the like. A similar survey in 1921 shows that 37 of 53 citations described an antiseptic as inhibiting microorganisms without necessarily killing them, whereas only 6 held that they functioned by killing. The mode of action of course may depend on such criteria as concentration used, time of contact, temperature, pH, nature of the organism, and organic matter present, and such conditions have been included in more restrictive definitions of many of these terms. For example, formerly under the Federal Food and Drug Act of 1938; mouthwashes, douches, and gargles that are allowed to remain in contact with the tissue for short periods were permitted to be labeled as antiseptics only if they destroy organisms in the dilutions recommended for use. The FDA (1974) specified clinical verification of infection prevention.

STERILIZATION

Sterilization is the act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms.

Comments

Because of the misuse of this term, the Council on Pharmacy and Chemistry of the American Medical Association (1936) officially went on record stating that sterilization was intended to convey an absolute meaning, not a relative one. A substance cannot be partially sterile. Nevertheless, the word is still used in the partial sense, as noted by Lawrence (1968), for mechanically filtered solutions called "sterile" although, while being free of bacteria and fungi and their spores, they may contain active viruses that are not removed by the filter.

Because it cannot be known with certainty whether all microorganisms of all types have been killed because we may not even be aware of the existence of some species or have media suitable for culturing all organisms, and

furthermore, because we can never prove the achievement of a negative absolute (species once claimed to be extinct occasionally turn up), it becomes more practical, as pointed out by Bruch and Bruch (1971), to use a process definition of sterilization. By this definition, sterilization is the process by which living organisms are removed or killed to the extent that they are no longer detectable in standard culture media in which they previously have been found to proliferate. According to this definition, both the process used to achieve sterility and the methods for testing for it are equally important.

Microorganisms of the same species vary in their resistance to chemical and physical agents, and if even one of a billion organisms survives, there is the possibility of rapid multiplication and contamination of the substance. Although, as stated, the term *sterile* implies an absolute, in practice it becomes a matter of probability whether sterility has been achieved. Different operations require different degrees of certainty of sterility, as measured by the percentage reduction or number of logarithmic reduction times (D values) in initial count brought about by the treatment.

OTHER DEFINITIONS

Algicide: A substance that kills algae, unicellular chlorophyll-containing plants.

Antibiotic: An organic chemical substance produced by microorganisms that has the capacity in dilute solutions to destroy or inhibit the growth of bacteria and other microorganisms; used most often at low concentrations in the treatment of infectious diseases of humans, animals, and plants. It is usually used as a chemitherapeutant and must be low in toxicity while effective against microorganisms.

Antimicrobial agent: Any agent that kills or suppresses the growth of microorganisms.

Asepsis: Prevention from contamination with microorganisms. Includes sterile conditions in tissues, on materials, and in rooms, as obtained by excluding, removing, or killing organisms.

Bactericide: An agent that kills bacteria. This term is applied to chemical agents that kill all bacteria, both pathogenic and nonpathogenic, but not necessarily bacterial spores. It is used for both living tissue and inanimate objects. It differs from *germicide* in that is does not include fungi, viruses, and other microorganisms that are not bacteria; it may kill them, but does not claim to do so.

Bacteriostat: An agent, usually chemical, that prevents the growth of bacteria but that does not necessarily kill them or their spores. Similarly, a *fungistat* and a *biostat* refer to the prevention of growth of fungi and of all living organisms, respectively. Sometimes the only difference as to whether a chemical is bacteriostatic or bactericidal depends on the conditions of application, such as time, temperature, or pH.

Bioburden: The microbiologic load, that is, the number of contaminating organisms in the product before sterilization. For example, it is more difficult to sterilize a product heavily loaded with spores than one lightly loaded.

Biocide: A chemical or physical agent that kills all living organisms, pathogenic and nonpathogenic. *Microbiocide* specifies an agent that kills microorganisms. Because a *biocide* kills spores as well as vegetative cells, it is presumably a sterilizing agent. The definition includes all species of living organisms, micro and macro, but the term is commonly used with reference to microorganisms. The term is usually used in nonmedical applications.

Biodeterioration: The deterioration of valuable materials due to biologic activity. The vectors are usually microbiologic but may be insects, rodents, higher animals, or plants. Deterioration may be noted by the breakdown of the material, biodegeneration, as in decay of timber, or aesthetic depreciation, as in staining of works of art.

Biologic indicator (BI): A standardized preparation of bacterial spores on or in a carrier serving to demonstrate whether sterilizing conditions have been met. Spores of different organisms are used for different methods of sterilization.

Chemisterilant: A chemical used to kill all microorganisms, including spores.

Chemitherapeutant: A chemical used inside the body that kills or suppresses microorganisms to control disease, such as quinine taken for malaria control or sulfa drugs for various microbial diseases.

Commensals: Microorganisms living on or within another organism, but not causing injury to the host under normal circumstances.

Contamination: The introduction of microorganisms into tissues or sterile materials.

D value, D_{10} value, or decimal reduction time (DRT): This value is defined as the time required to inactivate 90% of the cells present or to reduce the microbial population to one-tenth its number, that is, a one-logarithm reduction.

Decontamination: Disinfection or sterilization of infected articles to make them suitable for use. In a more general sense, decontamination is the process of freeing a person or object from potentially harmful material. This material can be infectious microorganisms, harmful insects, or toxic or radioactive chemicals.

Degerming agent: A disinfecting agent.

Disinfestation: Extermination or destruction of insects, rodents, or other animal forms that transmit disease, which may be on a person or his or her belongings, clothing, or surroundings.

Droplet nuclei: Particles of 5 μm diameter or less that are formed by dehydration of airborne droplets and are capable of air dispersal.

F value: The time in minutes required to kill all the spores in suspension at a temperature of 121°C or 250°F. By calculating and converting the temperature–time equivalents of the F value during the heating up and cooling down part of the sterilization cycle, and adding them together, the holding time at sterilization temperature may thereby be reduced and the product subjected to less heating than otherwise.

Fomites: Inanimate objects that may transmit infectious microorganisms (e.g., toilet seats, toothbrushes, silverware).

Fumigation: Exposure of an area or object to disinfecting, insecticidal, or vermin-killing fumes.

Fungicide: An agent that kills fungi. This term is associated with chemicals and includes substances applied to both living tissue and inanimate objects. Unlike the definition of *bactericide,* no mention is made to exclude spores; therefore, the assumption should be made that fungus spores are also killed or inactivated.

Fungitoxic: An agent is termed *fungitoxic* if it inhibits but does not necessarily kill fungi. It is equivalent to the term *fungistatic.*

Germicide: An agent that destroys microorganisms, especially pathogenic organisms. The word *germ* is a popular one and has been used to form the convenient term *germicide,* which finds extensive use in the technical as well as the popular literature. As commonly used, the term is associated with the death of all disease-producing microorganisms, but, like the word *disinfectant,* to which it is similar, it does not necessarily include the capability of destroying bacterial spores. Because *germicide* is a popular term, it is often misused to include sporicides, as in the phrase "high-level germicides," which includes sporicides like ethylene oxide. Use, however, eventually determines meaning, and if a word is generally used in a certain way, its meaning will change to conform to its common use, regardless of authority's efforts to the contrary. This seems to be occurring with the term *germicide.* It applies to use on both living tissue and inanimate objects. It refers to chemical agents, but the adjective *germicidal* may refer to physical agents, such as *germicidal* lamps.

Gnotobiotics: The science of rearing laboratory animals where the microflora and microfauna are known and there is no contamination from the outside.

Heat shock: Sublethal heat treatment to induce spore germination and destroy vegetative microorganisms.

High-level disinfectant: An agent capable of killing bacterial spores when used in sufficient concentration under suitable conditions. It is therefore expected to kill all other microorganisms.

Holding time: The time for which articles in a sterilizer must be held at a specified temperature to ensure that sterilization is attained.

Inactivation: Removal of the activity of microorganisms by killing or inhibiting reproductive or enzyme activity. When referring to an antimicrobial agent, *inactivation* means neutralizing its activity by any means.

Infection: The growth of microorganisms in a host.

Intermediate-level disinfectant: An agent that destroys all vegetative bacteria, including tubercle bacilli, lipid and some nonlipid viruses, and fungus spores, but not bacterial spores.

Isolator: An enclosure such as a plastic bag that is attached to a patient's body during surgical procedures to prevent contamination by infective agents. An isolator may also be used to prevent invasion by microorganisms in sterility testing procedures or rearing germ-free animals.

Laminar airflow: A system of parallel flows of air, horizontally or vertically, to reduce the chance of microbial contamination and infection, such as is used in hospital operating rooms and rooms of immunosuppressed patients.

Low-level disinfectant: An agent that destroys all vegetative bacteria except tubercle bacilli, lipid viruses, some nonlipid viruses, and some fungus spores, but not bacterial spores.

Nosocomial: Hospital-acquired infections. Infections not present or incubating before admittance to the hospital but obtained during the patient's stay in the hospital.

Ovicide: A substance that kills the eggs of very small infectious animals, such as the eggs of tapeworms.

Pasteurization: A process developed by Louis Pasteur of heating milk, wine, or other liquids to 60°C to 100°C (or the equivalent) for approximately 30 minutes to reduce significantly or kill the number of pathogenic and spoilage organisms.

Pathogen: Any disease-producing microorganism. In current circumstances, in which drugs, radiation, or the acquired immunodeficiency syndrome may cause immunosuppression, the distinction between pathogens and nonpathogens may no longer be significant.

Planktonic: Describes growth of microbiologic organisms dispersed in solution, as in the case of free-swimming plankton.

Preservation: The process by which chemical or physical agents prevent biologic deterioration of substances.

Prions: Little-understood, virus-like infectious agents that cause serious diseases of humans and animals. Prions are thought to differ from viruses by containing neither DNA nor RNA, only protein. They are extremely resistant to inactivation by heat and disinfecting agents.

Prophylactic: An agent that contributes to the prevention of infection and disease. This word is wide in scope. It is used for rubber condoms and antiseptic lotions and may even refer to fresh air and a nutritious diet when these serve to ward off disease.

Pyrogens: Fever-producing agents: endotoxins from the outer membranes of gram-negative bacteria, composed of complex lipopolysaccharide molecules.

Sanitizer: An agent that reduces the number of bacterial contaminants to safe levels as judged by public health requirements. It is commonly used with substances that are applied to inanimate objects. This term is associated with the cleaning of eating and drinking utensils and dairy equipment and is restricted to cleaning operations, as in the case of a *detergent sanitizer,* which combines both cleaning and antibacterial properties. According to the official sanitizer test, a sanitizer is a chemical that kills 99.999% of the specific test bacteria in 30 seconds under the conditions of the test.

Sessile: Describes growth of microorganisms attached to a body, not freely dispersed in planktonic growth.

Spores: The thick-walled resting cells produced by some bacteria and fungi that are capable of survival in unfavorable environments and are more resistant to antimicrobial agents than vegetative cells.

Sporicide: An agent that destroys microbial spores, especially a chemical substance that kills or inactivates bacterial spores. The term is commonly used in reference to substances applied to inanimate objects. Because spores are more resistant than vegetative cells, a *sporicide* would be a sterilizing agent, killing such resistant forms as amoebic cysts. However, the Association of Official Analytical Chemists (AOAC) (1987) makes a distinction between a *sporicide* and *sterilant,* requiring a more stringent test for a sterilant, thus: "For sporicidal claims, no more than 2 failures can be tolerated in this 120 carrier trial. For sterilizing claims, no failures can be tolerated."

Sterile: Free from living microorganisms.

Sterility assurance level (SAL): Because absolute sterility cannot be ensured, the SAL is the indication that no greater than the predetermined number of viable microorganisms can exist in a product, based on the intended use of a product and the risk determined to be acceptable.

Sterilization time: The time sterilizing conditions are maintained in a steam, hot air, or gas sterilizer.

Thermal death time: The time required to kill all spores at a specified temperature.

Tyndallization: A process of heat treatment for killing microorganisms by which the treated material is heated to less than sterilization temperatures, then allowed to cool and stand to incubate any spores that would germinate. This process is successively repeated for several days to destroy all vegetative forms and germinated spores.

Vegetative cells: Microbial cells that are in the growth and reproductive phase of the growth cycle.

Viable: Describes microorganisms capable of reproduction under favorable circumstances.

Viroids: Virus-like infectious agents that produce diseases in higher plants. They differ from true viruses in that they are believed to contain no protein, only low–molecular-weight RNA.

Virucide: An agent that destroys or inactivates viruses to make them noninfective, especially a chemical substance used on living tissue. The word *virucide* is a misnomer because the ending "cide" means *kill,* and the virus, by itself, is not a living entity. Thus, we do not say a virus is killed, but that a virus is inactivated. The spelling *viricide* is sometimes used, but is not preferred.

Z value: A measure of the way the D value changes with temperature for a particular organism. It may be considered the slope of the logarithm of the D value against temperature and the number of degrees to change the D value by a factor of 10. It is useful for comparing the death rate of spores with the destructive effect on the product over an equivalent temperature range.

OFFICIAL DEFINITIONS OF THE FDA AND EPA

The following are official definitions of the U.S. EPA and FDA. Although many of these definitions essentially duplicate those already given, they are presented here because they are the definitions of regulatory agencies, and in a legal dispute the understanding that a seemingly unimportant word or phrase may convey can be of great significance.

FDA (1997)

Bioburden (microbial load): The number and types of viable microorganisms with which an item is contaminated; also known as "bioload" or "microbial load" [Association for Advancement of Medical Instrumentation (AAMI), 1995].

Cleaning (or precleaning): The removal, usually with detergent and water, of adherent visible soil, blood, protein substances, and other debris from the surfaces, crevices, serrations, joints, and lumens of instruments, devices, and equipment by a manual or mechanical process that prepares the items for safe handling and/or further decontamination (AAMI, 1995).

D Value (D_{10}): Decimal reduction value. The exposure time required under a defined set of conditions to cause a 1-logarithm or 90% reduction in the population of a particular microorganism (AAMI, 1995).

Death rate curve (or survivor curve): The graphic representation of the microbial death rate kinetics for a specific microbicidal agent on a defined microbial population (AAMI, 1995).

Decontamination: According to the Occupational Safety and Health Administration (OSHA), "the use of physical or chemical means to remove, inactivate, or destroy blood-borne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal." [29 CFR 1910.1030] In common usage, *decontamination* generally refers to all pathogens (microorganisms capable of producing disease

or infection), not just those transmitted by human blood (AAMI, 1995).

Disinfectant: A chemical agent that eliminates a defined scope of pathogenic organisms, but not necessarily all microbial forms (e.g., bacterial endospores) (Rutala, 1990).

Disinfection: The destruction of pathogenic and other kinds of microorganisms by thermal or chemical means. *Disinfection* is a less lethal process than *sterilization* because it destroys most recognized pathogenic microorganisms, but not necessarily all microbial forms, such as bacterial spores. Disinfection processes do not ensure the margin of safety associated with sterilization processes (AAMI,1995).

Germicide: An agent that destroys microorganisms, especially pathogenic organisms. Other terms with the suffix "-cide" (e.g., virucide, fungicide, bactericide, sporicide, tuberculocide) indicate an agent that destroys the microorganism identified by the prefix (Block, 1991).

High-level disinfectant: A germicide that inactivates all microbial pathogens, except large numbers of bacterial endospores, when used according to labeling (Rutala, 1990; Spaulding, 1971, 1972).

Inorganic and organic load: The naturally occurring or artificially placed inorganic (e.g., metal salts) or organic (e.g., proteins) contaminants on a medical device before exposure to a microbicidal process.

Medical device (as defined by the Food, Drug, and Cosmetic Act): An instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is (a) recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them; (b) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in humans or animals; or (c) intended to affect the structure or any function of the body of humans or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of humans or other animals and which is not dependent on being metabolized for the achievement of any of its principal intended purposes.

Minimum effective concentration (MEC): The minimum concentration of a liquid chemical germicide that achieves the claimed microbicidal activity as determined by dose–response testing.

Minimum recommended concentration (MRC): The minimum concentration of a liquid chemical germicide at which efficacy has been demonstrated.

Process residue: The substance remaining on a medical device after exposure to a decontamination, disinfection, or terminal sterilization process.

Spaulding classification: A strategy for reprocessing contaminated medical devices. The system classifies medical devices as critical, semicritical, or noncritical based on the risk from contamination on a device to patient safety. The system also establishes three levels of germicidal activity (high, intermediate, and low) for disinfection strategies with the three classes of medical devices (Spaulding, 1971, 1972).

Spore (or endospore): The dormant state of an organism, typically a bacterium or fungus, that exhibits a lack of biosynthetic activity and reduced respiratory activity, and has resistance to heat, radiation, desiccation and various chemical agents.

Sterilant: An agent that destroys all viable forms of microbial life to achieve sterilization.

Sterile: The state of being free from all living microorganisms; in practice, usually described as a probability function, such as the probability of a surviving microorganism being one in a million (AAMI, 1995).

Sterility assurance level (SAL): The probability of survival of microorganisms after a terminal sterilization process, and a predictor of the efficacy of the process (AAMI, 1995).

Sterilization: A process intended to remove or destroy all viable forms of microbial life, including bacterial spores, to achieve an acceptable sterility assurance level (AAMI, 1995).

Total-kill end point analysis: A bracket verification test to confirm the established end point of the germicidal contact time.

Unit: A specified substrate or carrier on which a specified number of test organisms is inoculated. A unit may be a specified volume, weight, or surface area. For example, a unit could be specified as a test tube or Petri plate, an entire device, a component of a device (if the device must be disassembled before sterilization or disinfection), or a portion of a device.

Verification: Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled [Section 820.3 of the FDA Quality System Regulation (FDA, 1990)].

Vegetative state: An active growth phase of an organism.

FDA (1994)

Antiseptic drug: The representation of a drug, in its labeling, as an antiseptic shall be considered to be a representation that it is a germicide, except in the case of a drug purporting to be, or represented as, an antiseptic for inhibitory use as a wet dressing, ointment, dusting powder, or such other use as involves prolonged contact with the body.

Broad-spectrum activity: A properly formulated drug product, containing an ingredient included in the monograph, that possesses in vitro activity against the microorganisms listed in §333.470(a)(1)(ii), as demonstrated by in vitro minimum inhibitory concentration determina-

tions conducted according to methodology established in §333.470(a)(1)(ii).

Health-care antiseptic: An antiseptic-containing drug product applied topically to the skin to help prevent infection or to help prevent cross contamination.

Antiseptic handwash or health-care personnel handwash drug product: An antiseptic-containing preparation designed for frequent use; it reduces the number of transient microorganisms on intact skin to an initial baseline level after adequate washing, rinsing, and drying; it is broad spectrum, fast acting and, if possible, persistent.

Patient preoperative skin preparation drug product: A fast-acting, broad-spectrum, and persistent antiseptic-containing preparation that significantly reduces the number of microorganisms on intact skin.

Surgical hand scrub drug product: An antiseptic-containing preparation that significantly reduces the number of microorganisms on intact skin; it is broad spectrum, fast acting, and persistent.

FDA (1974)

Antimicrobial soap: A soap containing an active ingredient with in vitro and in vivo activity against skin microorganisms.

Health-care personnel handwash: A safe, nonirritating preparation designed for frequent use that reduces the number of transient microorganisms on intact skin to an initial baseline level after adequate washing, rinsing, and drying. If the preparation contains an antimicrobial agent, it should be broad spectrum, fast acting, and, if possible, persistent.

Patient preoperative skin preparation: A safe, fast-acting, broad-spectrum antimicrobial-containing preparation that significantly reduces the number of microorganisms on intact skin.

Skin antiseptic: A safe, nonirritating, antimicrobial-containing preparation that prevents overt skin infection. Claims stating or implying an effect against microorganisms must be supported by controlled human studies that demonstrate prevention of infection.

Skin wound cleanser: A safe, nonirritating liquid preparation (or product to be used with water) that assists in the removal of foreign material from small superficial wounds and does not delay wound healing.

Skin wound protectant: A safe, nonirritating preparation applied to small cleansed wounds that provides a protective (physical and/or chemical) barrier and neither delays healing nor favors the growth of microorganisms.

Surgical hand scrub: A safe, nonirritating, antimicrobial-containing preparation that significantly reduces the number of microorganisms on the intact skin. A surgical hand scrub should be broad sp ctrum, fast acting, and persistent.

EPA (1999)

Algicide: Any substance, or mixture of substances, that kills or effectively reduces the number of living algae in water.

Antifoulant: Any agent that is used to prevent the fouling of underwater structures.

Antimicrobial: The property of any pesticide to prevent, destroy, or mitigate any bacteria, pathogenic fungi, or viruses in any environment except those on or in living humans or other animals; those on or in living plants or the substrate in which they grow; and those on or in processed food, beverages, or pharmaceuticals including cosmetics. Antimicrobial products include sterilizers, disinfectants, virucides, tuberculocides, algicides, sanitizers, bacteriostats, and fungicides used against fungi that are pathogenic for humans or other animals. The term *antimicrobial* is too general to describe meaningfully a specific level of anitmicrobial activity.

Antiseptic: An agent that opposes sepsis, putrefaction, or decay by preventing or arresting the growth of microorganisms. Antiseptic products are applied on or in the living body of humans or other animals. Antiseptics are regulated by the FDA under the Federal Food, Drug and Cosmetic Act. Concurrent jurisdiction (under an interagency agreement) is exercised by the EPA and the FDA over products identified as both pesticides and antiseptics or drugs, when such products are recommended for use on both inanimate surfaces (such as dairy equipment) and on the living body of humans or animals (such as hand sanitizers, udder washes, teat dips, and hatching egg treatments).

Bacteriostat: An agent that inhibits the growth of bacteria in the inanimate environment in the presence of moisture, and in so doing, may or may not affect the viability of organisms.

Deodorizers: Chemical agents of two basic types: (a) those that prevent or delay the formation of bacterial odors by acting on microorganisms which produce them; and (b) those that mask, chemically destroy, or neutralize odors. Only those deodorizers that are described as type (a) are subject to regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Disinfectant: An agent that eliminates a specific species of infectious or other undesired microorganism, but not necessarily bacterial spores, in the inanimate environment only. The term *disinfection* connotes the combating of an infection, whereas infection involves only living plants and animals. For the purposes of this group of guidelines, the environment is considered contaminated, not infected, and the terms *germicide* and *bactericide* are synonyms for the word *disinfectant*.

Fungicide: An agent that destroys fungi (including y asts) and/or fungal spores pathogenic to humans or other animals in the inanimate environment.

Microbicide and biocide: Any substance, or mixture of substances, that effectively reduces the number of living

microorganisms (specifically: algicide—algae; bactericide—bacteria; fungicide—fungi; slimicide—slime-forming microorganisms). (Note: the terms *bactericide* and *fungicide* as used in conjunction with the term *microbicide* are related to industrial uses and are not related to products represented or defined as disinfectants.)

Microbiologic water purifier: Any unit, water treatment product, and/or system that removes, kills, or inactivates all types of disease-causing microorganisms from the water, including bacteria, viruses and protozoan cysts, so as to render the processed water safe for drinking. A microbiologic water purifier must treat or remove all types of challenge organisms to meet standards specified in the Guide Standard and Protocol for Testing Microbiological Water Purifiers.

Microbistat: Any substance or mixture of substances that effectively controls, or temporarily prevents, the growth of microorganisms (see *bacteriostat*).

Nonformulated tincture uses pattern: Refers to the addition of alcohol to a pesticide product as a diluent (instead of water) by the user, at the time of use. The intent of this practice is to provide increased efficacy of the registered pesticide by mixing it with another pesticidally active chemical that is not registered and is not marketed in conjunction with the registered pesticide.

Preservative: An agent that inhibits the growth of microorganisms capable of causing biologic deterioration of a substance(s)/material(s).

Sanitizer: An agent that reduces contaminants in the inanimate environment to levels considered safe as determined by Public Health Ordinance, or that reduces the bacterial population by significant numbers where public health requirements have not been established. Sanitizers meeting Public Health Ordinance requirements are generally used on food contact surfaces and are recognized as sanitizing rinses.

Sporicide (and variants thereof): A product that destroys or eliminates all forms of microbial life, including their spores, in the inanimate environment. For purposes of this subpart, *sporicide* and *sterilizer* are synonymous.

Sterilizer: An agent that destroys or eliminates all forms of microbial life in the inanimate environment, including all forms of vegetative bacteria, bacterial spores, fungi, fungal spores, and viruses.

Tuberculocide: An agent that destroys or irreversibly inactivates tubercle bacilli in the inanimate environment.

Virucide: An agent that destroys or irreversibly inactivates viruses in the inanimate environment.

ORIGIN OF TERMS

It might be of interest to examine the origin of a few of the terms we use, now that we have considered their meanings. The terms follow closely the history of disinfection, which is presented in Chapter 1. Some are as old as the English language; others are quite recent. The word *disinfectant* was first recorded in writing in 1598, with the meaning "to cure, to heale," but in 1658 it was used in the more modern sense, to remove infection: "They use to make great fires, where there is household stuffe of men that died of the Pestilence to dis-infect them." [See the *Oxford English Dictionary (OED)*, 1989, for references to quotations in this section.] In those days, it was believed that contagious diseases arose from effluvia, a flowing-out of invisible particles from the diseased person or corpse, or from miasmas, which were noxious disease-bearing exhalations from putrefying organic matter emanating from damp, unhealthful places like malarial swamps. Miasmas were referred to in medical writing as early as 1665. The word *disease*, coming from the French and meaning absence of ease, or discomfort, appeared in English about 1330 and was used by Chaucer in 1386. Chaucer was aware of the spread of infection by such means as coughing, for in 1386 he wrote, "Hoold cloos thy mouth . . . Thy cursed breeth infecte wole us alle." ("Hold closed your mouth . . . your cursed breath will infect us all.") The word *antiseptic* is credited to John Pringle, whose experiments in 1750 are described in Chapter 1. He was familiar with the word *septic*, first recorded in 1605, which means "putrefying." As a physician, he had written "The miasma or septic ferment being received into the blood," and when he found chemicals that prevented putrefaction, the word *antiseptic* was generated.

Although Leeuwenhoek first saw bacteria in 1676, the term was not recorded in use until 1847 to 1849. Leeuwenhoek just referred to "the little animals" or, in English, *animalcules*, a term first recorded in 1599 to refer to any small animal and in 1677 to mean those seen only under the microscope. It should be recognized that the terms discussed in this section may have been in oral use much earlier, but the written record gives the only dependable dates. After *bacteria*, we find Tyndall using the adjective *bactericidal* in 1878; *bactericide* follows in 1884 (the year that the word *bacteriology* appeared): "Permanganate of potash is not a bactericide of great activity."

The familiar word *microbe* did not arise by spontaneous generation, despite the strong belief by many respectable scientists in that mechanism of origin of microorganisms. It was the invention of the French surgeon, Charles Sédillot, an admirer of Pasteur and one of the first French physicians to apply Lister's antiseptic method. He wanted a word that would include bacteria and all the other tiny organisms that could be seen only with the microscope. The philologists criticized the word because its Greek root suggested an animal with a short life rather than an infinitesimally small animal. But with terminology for the tiny creatures in an uncertain state, and terms like *bacteridites* being used, Pasteur adopted the term and it was accepted worldwide. It was in 1878

that Sédillot presented his paper with the new word. By 1880 we had *microorganism*, by 1885 *microbiologists* and *microbiocide* in *Science* and *The British Medical Journal*, respectively, and the field of *microbiology* in 1888 in *Popular Science Monthly*. The adjective *microbiocidal* did not arrive until 1897.

The word *fungus* goes back much further because mushrooms and other fungi do not require a microscope to be seen. Fungus, spelled *fungous*, first appeared in the English language in 1420 and was used to describe a human infection in 1674 to 1677. The word *fungicide*, however, did not show up until 1889, when it appeared in *The Voice*, a New York City newspaper. It is probable that earlier use in technical publications was not uncovered by the *OED* researchers. As in the case of *bactericidal*, the adjective *germicidal*, in a medical journal, preceded in 1880 the noun *germicide*, in 1881, in the London *Times*. The word *germ*, in relation to a smallpox infection, was printed in 1803. *Germifuge* is listed in one medical dictionary as an obsolete term for disinfectant, but I could find no reference to its actual use.

In reading an article on disinfection written in the early 1800s, I was surprised to see the word *virus* used in connection with a disease. This was many years before bacteria were known to cause disease, and viruses could not have been seen even with the microscope. On looking into the matter, I discovered that *virus* was the Latin word for *poison* and was used, as such, as early as 1599. Its use eventually became more restricted to a substance related to contagious disease, as in the 1728 description, "a corrosive or contagious pus." In 1880 Pasteur stated, "The virus is a microscopic parasite which may be multiplied by cultivation outside the body of an animal." The adjective *virucidal* was first noted in 1925, and to our surprise the *OED* does not contain the word virucide. The term *biocide*, to mean destruction of tissues of the human, was first recorded in 1947, and in 1963 Rachel Carson used biocide in her book *Silent Spring* to mean "genocide." In 1968, at a biodeterioration symposium, it was finally used in the way we define it, as referring principally to microorganisms, being a sterilizing agent. *Sterilization*, as in "sterilization by heat or organic liquids," was written in 1874, but the word *sterile* did not appear until 1877; in 1891, the steam *sterilizer* was mentioned in print. *Antibiotic* did not come into use until after penicillin, appearing in *The Lancet* in 1944. The term *sanitizer* appeared first in the *Journal of Milk and Food Technology* in 1950. The second mention of *sanitizer* listed by the *OED* was in 1968, and referred to the first edition of this book, where Chapter 4 gave methods for testing sanitizers and bacteriostatic substances. Thus, this book not only records the history of disinfection but itself has become part of that history.

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Housekeeping surfaces have the least potential for cross-contamination among health care personnel, patients, or medical equipment and instruments. Adequate safety levels can be achieved by maintaining these surfaces in a state of visible cleanliness by using water and a detergent or a hospital grade disinfectant/detergent designed for general housekeeping purposes (as indicated on the product label). Only in instances in which there has been a significant spill of blood or other potentially infectious body fluid or laboratory cultures should the added use of an intermediate-level chemical disinfectant be considered to ensure that such a surface is "safe."

The remainder of this chapter will consider disinfection or sterilization only in the context of "medical and surgical materials"; but it is clear that the choice of disinfecting methods rests largely with the judgment of the health care professional, particularly in the case of many semicritical and most noncritical instruments or devices. Many factors are involved in this decision-making process, including the manufacturer's instructions, how the item will contact the next patient, the physical configuration (cleanability) of the item, the type and degree of contamination after use, the physical or chemical stability of the item, and the ease or difficulty in removing (rinsing, aerating) the chemical agent after the necessary exposure time. Ideally, the manufacturer's instructions for use of the item should include detailed instructions for all aspects of reprocessing; in reality, this is not uniformly the case. Therefore, consistent and effective procedures for chemical disinfecting or sterilizing of medical and surgical materials often depend greatly on the knowledge and judgment of the responsible health care practitioner.

DEFINITION OF TERMS

Although the definitions of sterilization, disinfection, and antisepsis (Spaulding, 1972; Favero and Bond, 1993; Rutala, 1996; Widmer and Frei, 1999) have been accepted generally, it is common to see these terms misused. The exact distinction and the basic knowledge of how to achieve and monitor each state are important if the effective application of long-known principles is to be realized.

Sterilization

The term *sterilization* is one that students and professionals have memorized and recited seemingly forever. It can be the simplest or the most complex concept, depending on how it is viewed and how it is applied. The definition of sterilization can change depending on the user's viewpoint. We view this term somewhat like a hologram and define it in the context of *state* of sterilization, the *procedure* of sterilization, and the *application* of sterilization.

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms. This *state* of sterility is the objective of the sterilization procedure and, viewed in this context, the definition is categorical and absolute; that is, an item is either sterile or it is not. A sterilization *procedure,* on the other hand, cannot be categorically defined. Rather, a sterilization procedure is defined as a process, after which the probability of a microorganism surviving on an item is less than one in one million (10^{-6}). This is referred to as the *sterility assurance level,* and this approach is used by the medical device industry to sterilize large quantities of medical devices. Criteria used in the production and labeling of a sterile device include good manufacturing practices, validated sterilization process, sterility testing of a subsample of the batch subjected to the sterilization process, process controls, use of biologic indicators, quality control of materials, and poststerilization testing of devices for function.

The *application* of the sterilization process takes into account additional considerations. This approach involves the strategy associated with a particular medical device (or medical fluid) and the context of its degree of contact with patients. The example would be the use of the Spaulding classification system to determine the strategy for disinfection or sterilization.

Sterilization is the use of a physical or chemical procedure to destroy all microorganisms including large numbers of resistant bacterial spores. Historically, the development of a sterilization cycle involves the determination of a sterility assurance level by using a specific biological indicator and constructing an inactivation curve and obtaining a D value (see Chapter 6) This approach is illustrated in Fig. 43.1. The inactivation rate is linear, and the D value can be obtained for a given set of sterilization parameters. In practice, a survivor curve spanning six logs of inactivation can be determined by using quantitative spore assays. A six-log reduction is considered a *half-cycle.* An additional six logs of inactivation can b deduced by extrapolation of the linear survivor curve. This procedure is used to construct a sterilization cycle with a sterility assurance level of 10^{-6}, meaning that there is a probability of one chance in a million that one of the one million spores used in the starting challenge survived the sterilization cycle (Favero, 1998a) (see also Fig. 43.1). In the development of sterilization cycles for sterilizers, the approach is extraordinarily conservative (see Chapter 67). Manufacturers of sterilization systems, including low-temperature systems, employ a high degree of conservatism in designing sterilization cycles, including the following:

An assumption that the bioburden on a device is 10^6 and consists of bacterial spores most resistant to the process

In challenge tests, placement of spores in least accessible location in device

Use of spores contained in "soil"

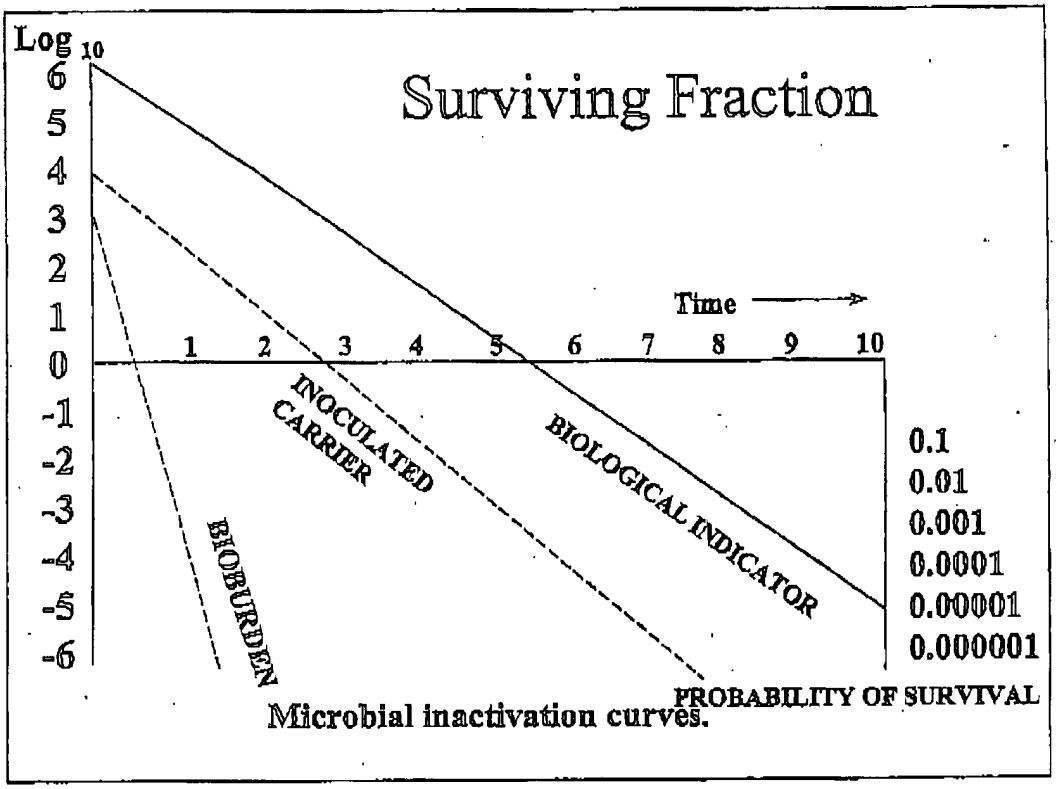

FIG. 43.1. The comparative resistance of bacterial spores used as biologic indicators and the naturally occurring bioburden exposed to a sterilization process.

Application of simulated use conditions
Documentation of six logs of kill at half-cycle
Documentation that a cycle produces six logs of kill with a 10^{-6} probability that one spore survives

In reality, however

Most medical devices have a bioburden of less than 10^3.
Gastrointestinal endoscopes (e.g., colonoscopes) have a bioburden of 10^8 to 10^9.
Cleaning reduces the bioburden by three to five logs.
The bioburden is composed of vegetative bacteria, viruses, and fungi, and less than 0.1% are spores, if any.

The spectrum of inactivation kinetic rates among experimental and in-use microbial challenges is illustrated in Fig. 43.2. In the design and validation of the sterilization cycle, a challenge of one million bacterial spores highly resistant to the process is used, and the survival curves reflect this conservative approach. The actual bioburden (Spaulding, 1939; Nystrom, 1981; Chan-Myers et al., 1997; Rutala et al., 1998) is less than a thousand and consists primarily of vegetative bacteria that are killed much more rapidly than are bacterial spores. The conservative nature of the sterilization cycles in conjunction with instrument and device cleaning results in a significant overkill and ensures a huge margin of sterilization success and a high degree of patient safety (Favero, 1993).

In the hospital, sterilization and disinfection specifically pertain to microorganisms that may exist on inanimate objects. Moist heat by steam autoclaving, ETO, and hydrogen peroxide gas plasma are the major sterilization systems used in hospitals. As will be discussed, however, numerous chemical germicides have been used for purposes of sterilization of heat-sensitive instruments and appear to be effective when used appropriately. In the United States, chemical germicides formulated as sterilants are almost never used to sterilize instruments. Rather, these germicides are used for (high-level) disinfection. Unfortunately, some health care professionals refer to almost any disinfection procedure as "sterilization," which leads to a degree of confusion that often becomes magnified with routine use. A good example is the use of short exposures to glutaraldehyde-based germicides for the disinfection of certain flexible fiberoptic endoscopes. Some practitioners refer to this practice as "sterilization" of endoscopes. For instance, a 2% glutaraldehyde solution is capable of sterilization but only after extended contact time in the absence of extraneous organic material. Unfortunately, flexible fiberoptic endoscopes are not physically capable of withstanding

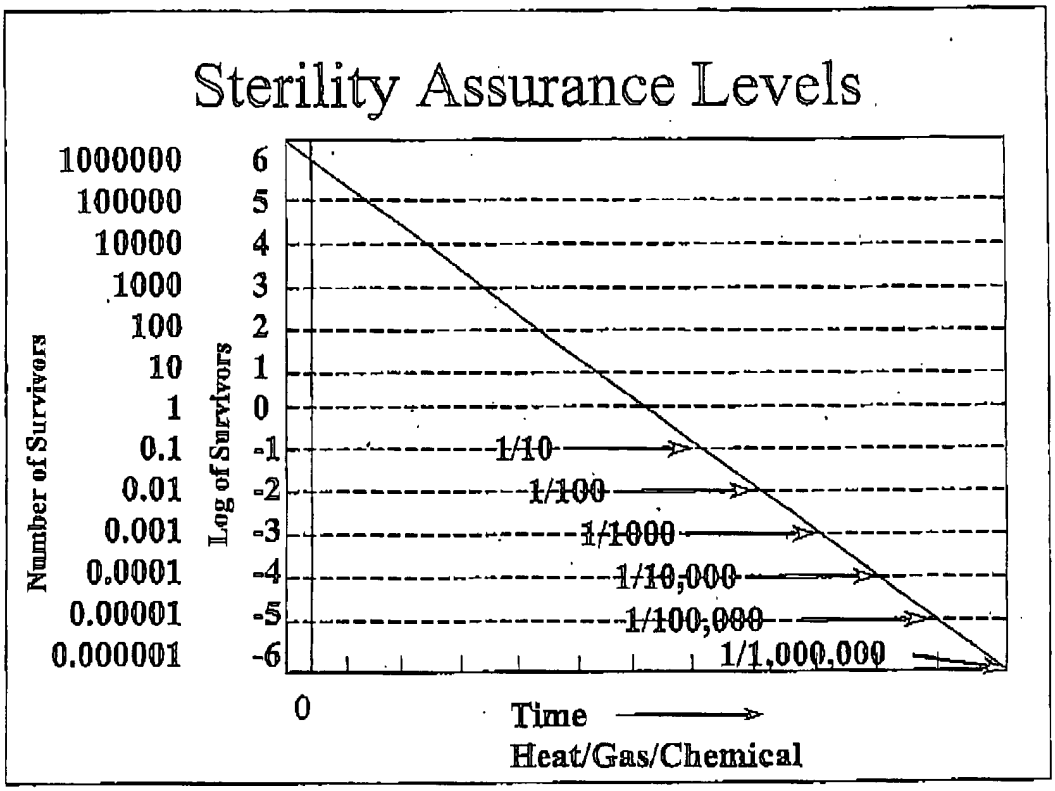

FIG. 43.2. Sterility assurance levels.

repeated immersion in fluid for 6 to 10 hours; in fact, most manufacturers state that repeated prolonged immersion will damage the instruments and recommend that routine immersion times not exceed 20 to 90 minutes. Thus, the procedure used for most flexible endoscopes is high-level disinfection and not sterilization, despite the fact that colloquially this procedure is often referred to in some hospitals or other health care facilities as "sterilization."

As mentioned, the state of absolute sterility is difficult to prove; as a result, it is common to define sterility in terms of the probability that a contaminating organism will survive treatment. For example, sterilizing processes are designed and often monitored using a high number (10^6 to 10^7) of dried bacterial spores, and *sterilization* is defined as that state in which the probability of any one spore surviving is 10^{-6} or lower. This rationale has been used to establish cycles for steam autoclaves, ETO, and hydrogen gas plasma sterilizers, and it produces a great degree of overkill as well as a quantitative assurance of sterilization. It is difficult to evaluate liquid chemical sterilization or disinfection processes by using these criteria unless the liquid chemical sterilant is part of a system and evaluated in the same manner as other sterilizers.

Disinfection

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. As can be seen by this definition, disinfection does not ensure an "overkill"; therefore, disinfection processes lack the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each of which may have a pronounced effect on the end result. Among these are the nature and number of contaminating microorganisms (especially the presence of bacterial spores), the amount of organic matter (e.g., soil, feces, blood) present, the type and condition of the medical and surgical materials to be disinfected, and the temperature. Accordingly, disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other. It should be noted that the acceptance of such a distinction is consistent with the abilities of certain nonsporicidal disinfectant solutions to destroy completely any microbial contamination on medical and

EXHIBIT B

Irradiation of Polymers

Fundamentals and Technological Applications

EDITED BY
Roger L. Clough and Shalaby W. Shalaby

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Chapter 17

Gas-Plasma Sterilization

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Low temperature hydrogen peroxide gas plasma has been developed as a new method of sterilizing medical products. The process has been shown to inactivate a broad spectrum of microorganisms, including resistant bacterial spores. A Sterility Assurance Level (SAL) of 10^{-6} has been demonstrated for the process utilizing Bacillus stearothermophilus spores, the most resistant organism tested. Material compatibility studies have shown that the process is compatible with a wide range of metallic and non-metallic devices. When compared to *r*-irradiation, the low temperature hydrogen peroxide gas plasma has been found to affect the surface properties, i.e., wetting properties, of some non-metallic devices but not the bulk physical properties. Functionality studies have also shown that heat and moisture sensitive electronic, optical and mechanical devices are not adversely affected by exposure to the process.

The common methods of sterilizing packaged medical products in a hospital environment have historically involved the use of steam or dry heat for heat tolerant medical devices, and ethylene oxide gas or formaldehyde gas for heat sensitive devices. In the industrial area, radiation sterilization, mostly involving gamma irradiation (Cobalt 60), has been used for over 35 years, and more recently sterilization with electron beam has become more widespread. However, due to the large investment required to install a gamma irradiation facility or an electron beam sterilization process, the use of these technologies have not moved from the industrial to the hospital setting. Additionally, the detrimental effects of ionizing radiation on the bulk properties of some nonmetallic materials has placed some limits on the general use of these technologies on medical devices that are intended to be reused and exposed to repeated sterilization processes.

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As the number of heat and moisture sensitive devices in the hospital has increased, especially in the diagnostic and less invasive surgery areas, so has the need for a rapid method of sterilizing these devices so that they can be reused many times each day. Ethylene oxide, the conventional method for sterilization heat sensitive devices, requires a long turn around time due to the extended aeration times required to remove the toxic ethylene oxide from sterilized items. In addition, ethylene oxide has been under increased regulatory pressures worldwide, due to the inherent toxicity of the ethylene oxide gas, and the detrimental effect of the chlorofluorocarbons used in the process on the earth's ozone layer. The use of formaldehyde gas has toxicological concerns similar to those expressed for ethylene oxide. A combination of all of these factors has accelerated the search for alternative methods of sterilizing heat and moisture sensitive materials. As a results of that search, Low Temperature Gas Plasma has emerged as a new technology capable of rapidly sterilizing sensitive medical devices.

Plasma Technology

Plasma is defined as a fourth state of matter, energetically distinguishable from solids, liquids, and gases. It can be produced through the action of either high temperatures, or electric or magnetic fields, and it is normally composed of a cloud of ions, electrons, and neutral species. Depending upon the environment in which it exists, i.e., field strength, interference, etc., the exact composition of the plasma will differ. For an ionized gas to be properly defined as a plasma, the number of positively and negative charged species present in the discharge must be approximately equal. This requirement is satisfied when the dimensions of the discharge gas volume, Λ, are significantly larger than the Debye length (*1*).

$$\lambda_D = \left| \frac{E_o K Te}{n e^2} \right|^{1/2}$$

The Debye length represents the distance over which a charge imbalance can exist. In the above equation, E_o is the permittivity of free space, K is the Boltzmann constant, Te is the electron temperature, n is the electron density, and e is the charge on the electron.

Plasmas can be conveniently divided into two categories. One category includes these plasmas that have the common characteristic of having temperatures in excess of 5000°K and are described as high temperature plasmas. While these plasmas exist in nature (i.e., in the sun or stars) and can be created in high pressure arcs or plasma jets, they are of no interest in plasma sterilization of medical devices due to the extreme temperatures involved.

The plasma utilized in plasma sterilization processes is in the second category known as glow discharge, or low temperature plasma. Low temperature plasmas, such as those found in neon lights or in sterilization processes, are created under vacuum conditions. These plasmas have average electron energies in the range of 1 to 10 eV and electron densities in the range of 10^9 to 10^{12} cm^{-3}. There is also a lack of thermal equilibrium in these plasmas between the electron temperature Te and the gas temperature Tg. The ratio of Te/ Tg is typically in the range of 10 to 100. For that reason, these plasmas have the unique properties of having electrons or other species that have sufficient energy to cause the rupture of molecular bonds while the temperature of atoms and molecules in the plasma are near ambient values. It is this characteristic that makes low temperature plasmas well suited to the sterilization of thermally sensitive materials.

Background of Plasma Sterilization

For over 25 years researchers have investigated the use of plasma technology to sterilize various materials and devices. One of the first reports on plasma sterilization was in a 1968 patent issued to Arthur D. Little Company (*2*) in which the use of a pulsed higher pressure/ high temperature plasma sterilization process was disclosed. A second patent issued in 1972 to Arthur D. Little (*3*) disclosed the first use of a low pressure, low temperature plasma process. This process utilized halogen gases to sterilize contaminated surfaces. Additional patents were issued in 1974 to Boeing Company (*4,5*) on the development of flow-through plasma sterilization systems for medical devices, in 1980 to Boucher (*6*) on a plasma process utilizing aldehydes in the gas phase, and in 1982 to Motorola (*7,8*) on sterilizing through sealed porous packaging and on the use of pressure pulsing of the plasma to enhance antimicrobial efficiency in diffusion restricted environment such as lumens.

In 1987, a patent was issued to Surgikos, Inc., a Johnson & Johnson Company (*9*), on the use of a low temperature gas plasma sterilization process utilizing hydrogen peroxide as the precursor chemical. One of the unique features of this process involved the use of a pretreatment stage to allow the hydrogen peroxide to diffuse through the materials and come in close proximity to the devices to be sterilized prior to the generation of the low temperature plasma. This feature allows for the efficient sterilization of packaged medical devices. The importance of this feature was discussed in a 1989 paper by Addy (*10*) on low temperature plasma sterilization. The first commercial plasma sterilization product, the STERRAD 100 sterilizer was developed based on this patented process.

In 1992, a patent was issued to Abtox, Inc. (*11*) on the use of another low temperature plasma sterilization process that also involves a pretreatment step. In this process, the pretreatment chemical involved a mixture of peracetic acid and hydrogen peroxide. The plasma is generated upstream by microwave discharge and also contains oxygen, hydrogen, and argon gases.

Although not all inclusive, these disclosures reflect the interest generated over the past 25 years on the application of low temperature gas plasma technology to the sterilization area. As the development of low temperature gas plasma sterilization has reached the commercialization stage, the interest in this area has continued to increase.

Generation of Low Temperature Plasma

Plasmas are normally referred to by the type of processor gas or vapor from which they are formed. For example, in the STERRAD Process, hydrogen peroxide and water molecules are initially introduced into a vacuum during the injection stage. The hydrogen peroxide and water molecules diffuse throughout the chamber and come in intimate contact with the materials to be sterilized during the diffusion stage, and the plasma that is generated during the plasma phase is known as a hydrogen peroxide plasma.

There are several different methods of generating low temperature gas plasmas. The most common methods, which are reviewed by Grill (*12*) and Bell, et al. (*13*) include direct current (DC), radio frequency (RF) and microwave (MW) power applied to a gas. RF systems are the most common and provide more flexibility from a design consideration. For example, RF plasmas can be sustained with internal as well as external electrodes, while DC discharges require the electrodes to be inserted inside the reactor and be in direct contact with the plasma. RF plasmas are also characterized by higher ionization efficiencies than DC plasma and can be sustained at lower pressures than DC or MW plasmas.

With RF induced plasma, one can utilize either a capacitively coupled system o an inductively coupled system for coupling the RF energy into the plasma. Different chamber and electrode designs can also be utilized such that samples t be processed will reside in either a high intensity or a low intensity electric fiel All of these factors can potentially affect the material compatibility of device sterilized in a plasma sterilization process.

When working with glow discharge processes, it is important that the prope safety precautions be observed. The STERRAD Sterilization Process utilizes a RF generated plasma operating at 13.56 MHz, a frequency approved by th Federal Communication Commission (FCC) for industrial application. The R power supply used to generate the low temperature gas plasma in the STERRA Sterilizer can only be turned on when the sterilization chamber door is closed an the chamber is under vacuum. In addition, the unit is shielded to couple wit FCC class A electromagnetic interference emissions standard. The STERRA Sterilization System also meets the IEC CISPR standard and the VDE 87 Electrical Standard, the world's most stringent standard for RF emissions.

Chemistry of Plasma Sterilization

The chemistry of a plasma sterilization process will depend upon the precursor gases or vapors from which the plasmas are generated. In the STERRAD Sterilization Process, which utilizes H_2O_2 in the pretreatment step, a limited number of decomposition products can be generated during the plasma stage due to the simplicity of the H_2O_2 molecule.

The steps involved in the STERRAD Sterilization Process are as follows: In the pretreatment phase, hydrogen peroxide is introduced into the low initial pressure of the chamber. The hydrogen peroxide evaporates and is allowed to diffuse throughout the sterilization chamber and thereby come into close proximity with the items to be sterilized. The hydrogen peroxide serves as a precursor for the generation of free radicals and other biologically active chemical species. In addition, the hydrogen peroxide vapor can also exert lethal effects of its own. In a highly simplified form, the reactions in plasma for which H_2O_2 serves as a precursor may be summarized as follows:

$$\text{H-O-O-H} \xrightarrow{e^-,\ 1-10\ eV} \text{HO}\cdot + \cdot\text{OH}$$

$$\text{HO}\cdot + \text{H-O-O-H} \longrightarrow \text{HOH} + \cdot\text{O-O-H}$$

$$\text{H-O-O-H} \xrightarrow{e^-} \text{H-O-O-H}^*$$

$$\text{H-O-O-H}^* \longrightarrow \text{H-O-O-H} + \text{visible and UV radiation}$$

The chain of events resulting in the low temperature hydrogen peroxide plasma is initiated by the accelerated electrons which result from the electric field imposed on the chamber by the RF system. This series of events is similar to those suggested to occur during the secondary reactions related to gamma irradiation, i.e. (*14*).

$$\text{H-O-H} \xrightarrow{\gamma\ 1.17-1.33\ MeV} \text{HO}\cdot + \cdot\text{H}$$

$$\text{H}\cdot + \text{O}_2 \longrightarrow \cdot\text{O-O-H}$$

$$\text{HO}\cdot + \cdot\text{OH} \longrightarrow \text{H-O-O-H}$$

$$2(\cdot\text{O-O-H}) \longrightarrow \text{H-O-O-H} + \text{O}_2$$

A major difference in these series of events involves the energies associated with the initiation of these reactions. In the low temperature gas plasma, electron energies are in the 1-10 eV range, while in gamma irradiation, the energies are in excess of 1 MeV. This difference in energies, which relate to th fact that low temperature plasmas are known to affect only a thin layer, a few atoms in depth, on the surface of non-metallic materials as compared to the bulk effects observed with gamma irradiation, will be discussed in section VII on effects of low temperature plasma sterilization on medical materials and devices. Other reactions also occur in the hydrogen peroxide plasma to produce reactive oxygen (O·) and hydrogen atoms, and oxygen and water as stable by-products.

$$\cdot\text{OH} + \cdot\text{OH} \longrightarrow \text{H}_2\text{O} + \text{O}\cdot$$

$$\cdot\text{OH} + \cdot\text{O} \longrightarrow \text{H}\cdot + \text{O}_2$$

$$\cdot\text{OH} + \cdot\text{O-O-H} \longrightarrow \text{H}_2\text{O} + \text{O}_2$$

During the plasma phase, the series of reactions depicted more or less continually take place under the influence of the RF energy. At the termination of the plasma phase of the cycle, any reactive species remaining recombine to form stable chemical species, predominately $H_2O + O_2$. Both metallic and nonmetallic medical substrates exposed to the low temperature hydrogen peroxide plasma STERRAD process have shown no increase in toxicological properties after exposure to repeated sterilization cycles.

The biological activity of many of the active species generated in hydroge peroxide plasma have been the objects of study by a number of researchers (*1*: *18*). The free radicals generated in the hydrogen peroxide plasma are known to b reactive with almost all of the molecules essential for the normal metabolism an reproduction of living cells, i.e., DNA, RNA, enzymes, phospholipids, etc. Whi an exact model of the mode or modes of action in the inactivation microorganisms by the STERRAD Sterilizer cannot be precisely establishe there exists in the hydrogen peroxide plasma process a biocidal environme which comprises many reactive species, i.e., free radicals, UV radiation, hydroge peroxide, etc., that are capable of inactivating microorganisms by chemic interactions at multiple biologically important reaction sites.

Efficacy

The efficacy of the STERRAD Sterilization System was established, in part, b demonstrating the ability of the system to kill a broad spectrum microorganisms selected for their known resistance to chemical and physic sterilants and to provide by universally accepted validation methods a Sterili Assurance Level (SAL) greater than 10^{-6} with highly resistant bacterial spore Additional tests were also conducted with actual medical products to confirm t validation studies conducted with the STERRAD Sterilizer.

Spectrum of Activity. By definition, a sterilization process must have the ability to inactivate a broad spectrum of microorganisms, including resistant bacterial spores. As previously noted, the hydrogen peroxide plasma process contains a number of reactive species known to be reactive with molecules essential for the normal metabolism and reproduction of microorganisms. Because of the number of reactive species involved and the ability of these species to react at multiple reaction sites in the microorganisms, one would expect that the hydrogen peroxide plasma process would exhibit broad spectrum antimicrobial activity.

Spectrum of activity studies were conducted against vegetative bacteria (including Mycobacteria), bacterial spores, yeasts, fungi, and viruses. In general, these organisms were chosen for their resistance to hydrogen peroxide, other chemical sterilants or ionizing radiation, as has been documented in scientific literature. Those organisms currently used to monitor steam, ethylene oxide and ionizing radiation sterilization processes were included in these studies. The reason for the selection of each test organism as well as the results of these tests are presented in Table I. All of the organisms shown in Table I were found to be efficiently killed by an abbreviated STERRAD System cycle consisting of 20 minutes of diffusion with 2 mg/liter of hydrogen peroxide and 5 minutes of plasma at a power of 300 watts. By comparison, the standard STERRAD Sterilization Cycle consists of 50 minutes diffusion with a minimum of 6 mg/liter of hydrogen peroxide and 15 minutes of plasma at 400 watts. As the STERRAD System cycle conditions were further reduced, bacterial spores were found to be the most resistant organism to the process. These results are consistent with those observed with other sterilization processes in which bacterial spores have been found to be more resistant to inactivation than other microorganisms.

The two virus tested, Poliovirus Type 1 and Herpesvirus Type 1, are representative of the two major classes of viruses, hydrophilic and lipophilic viruses, respectively. Of these two classes, the hydrophilic group normally exhibits the greater resistance to chemical sterilants, and poliovirus is known to be a highly resistance hydrophilic virus. The log 10 virus titers of 3.98, 3.20 and 2.84 represent the minimum concentration of viruses in these tests. Due to the nature of the virucidal test, a minimum virus concentration is determined but the actual concentration is not. In all virucidal tests there was no infectivity obtained after exposure to the highly abbreviated STERRAD System Cycle. This shows that even an abbreviated STERRAD System Cycle is capable of inactivating both hydrophilic and lipophilic viruses.

Validation of 10^{-6} Sterility Assurance Level (SAL). Well established and universally accepted methods exist for the validation of sterilization processes. For example, in the Association for the Advancement of Medical Instrumentation (AAMI), Standards and Recommended Practices, Volume 1: Sterilization (1992) (*19*), the procedures for the validation of steam and ethylene oxide sterilization processes are specified. Implicit in these test methods is the demonstration of at least a 10^{-6} sterility assurance level (SAL) for the sterilization process.

Table I
SPECTRUM OF ACTIVITY
VEGETATIVE BACTERIA, SPORES AND FUNGI

MICROORGANISM	TYPE	INTEREST IN TESTING	CONTROL[1]	RESULTS[2]
Bacillus stearothermophilus	Bacterial Spore	H_2O_2 Resistance; Steam Indicator Organism	2.04×10^6	0/9
Bacillus subtilis var. *niger (globigii)*	Bacterial Spore	H_2O_2 Resistance; EtO Indicator Organism	2.69×10^6	0/9
Bacillus pumilus	Bacterial Spore	Ionizing Radiation Resistance and Radiation Indicator Organism	1.82×10^6	0/9
Staphylococcus aureus	Gram Positive	H_2O_2 Resistance; Clinical Significance	2.82×10^6	0/9
Deinococcus radiodurans	Gram Positive	Ionizing Radiation Resistance	3.10×10^6	0/9
Pseudomonas aeruginosa	Gram Negative	Clinical Significance	1.32×10^6	0/9
Escherichia coli	Gram Negative	Clinical Significance	9.23×10^5	0/9
Serratia marcescens	Gram Negative	H_2O_2 Resistance; Clinical Significance	1.85×10^6	0/9
Moraxella osloensis	Gram Negative	Ionizing Radiation Resistance	3.14×10^6	0/9
Mycobacterium bovis	Acid Fast	Chemical Resistance; Clinical Resistance	4.20×10^6	0/9
Candida albicans	Yeast	H_2O_2 Resistance	3.95×10^6	0/9
Candida parapsilosis	Yeast	H_2O_2 Resistance; Clinical Significance	1.07×10^6	0/9
Trichophyton mentagrophytes	Filamentous Fungus	Clinical Significance	1.25×10^6	0/9
Aspergillus niger	Filamentous Fungus	H_2O_2 Resistance; Clinical Significance	1.46×10^6	0/9

1. Average titer recovered from nine samples
2. # Positive / # Tested

Continued on next page

Table I. Continued

VIRUSES

MICROORGANISM	TYPE	INTEREST IN TESTING	VIRUS TITER Log 10		INFECTIVITY
Poliovirus Type 1 (Brunhilde)	Hydrophilic	Chemical Resistance; Clinical Significance	Test 1 Test 2	≥3.98 ≥3.98	Not detected Not detected
Herpesvirus Type 1	Lipophilic	Clinical Significance	Test 1 Test 2	≥3.20 ≥2.84	Not detected Not detected

Sterilization is a probability function and a minimum SAL of 10^{-6} means that the probability of a bioburden microorganism surviving after exposure to the sterilization process is no greater than 10^{-6}. Or it can also be stated that the probability of having a non-sterile device after processing is less than one in one million when the sterilizer is used as directed. This definition for sterility of terminally sterilized products is well accepted in the scientific community.

Critical to the demonstration of the 10^{-6} SAL is the use of a consistent and reproducible biological monitor for evaluating the efficacy of the sterilization process. According to the AAMI guidelines the organism used in the biological challenge should be resistant to the sterilization process being monitored. The recommended biological challenge organisms for steam and ethylene oxide sterilization processes are Bacillus stearothermophilus and Bacillus subtilis var. niger, respectively. It is recommended that B. stearothermophilus be used at a population of 10^3 to 10^6 and that B. subtilis be used at a population of at least 10^3 and that it is typically used at a population of 10^6.

The STERRAD Sterilizer was validated by the classical overkill method of validation (AAMI Standards and Recommended Practices, Vol. 1 : Sterilization 1992). The overkill approach is based on the premise that the sterilization process will inactivate a given population of a spore challenge that is resistant to the sterilization process and provide an additional safety factor. The method is called "overkill" because the cycle conditions established to kill the resistant spore challenge, with an additional safety factor, are far more severe than those required to inactive the product bioburden. For example, for ethylene oxide sterilization, the typical spore challenge is 10^6 spore of B. subtilis, and an overkill cycle would provide a 6 log reduction of the microbial challenge at one-half of the sterilization cycle exposure time. This provides for a safety factor since the complete cycle would provide a theoretical 12 log reduction of the B. subtilis spores that are more resistant to the ethylene oxide sterilization process than normal bioburden organism.

Two methods are specified in the AAMI standards for detecting the number of organisms present on a biological indicator used in the validation of a steam or ethylene oxide sterilization process. The first method in which the number of viable organisms on the biological indicator is counted or enumerated is generally known as the survivor curve, count reduction or plate count method. The surviving number of microorganisms is evaluated using standard microbiological plating techniques, at fractional sterilization cycle exposure times. The second method, which is known as the quantal analyses, fraction-negative analyses, or sterility test method, involves placing the biological indicator in a broth media and evaluating for a growth/no growth response. Replicate units are exposed to fractional sterilization cycles, cultured, incubated, and scored for growth (positive) or no growth (negative). The data can be used to estimate the number of surviving organisms per unit using the most probable number analysis of Halvorson and Ziegler (*20*). The STERRAD Sterilization System was validated by the universally accepted overkill procedure utilizing both the survivor curve and fraction negative test methods.

Selection of Validation Organism. Before validating the STERRAD Sterilization Cycle, studies were conducted to determine which organism exhibited the greatest resistance to the hydrogen peroxide plasma process. Since spectrum of activity tests had shown that bacterial spores were the most resistant of the organisms tested, tests were conducted on four bacterial spores used to validate comparable sterilization systems to determine their relative resistance to the STERRAD Process. In these tests the test organism was inoculated onto paper strips which presented a greater challenge than with medical material substrates because paper tends to absorb hydrogen peroxide thus competing with the spores for the available hydrogen peroxide in the system. The paper strips were placed in diffusion restricted tubular test pieces that were then placed in standardized hospital trays of instruments.

The test method used involved varying the amount of diffusion time in the cycle holding all other components of the cycle fixed at their full value. This method was used because the biological challenge was placed in the most diffusion restricted location in the validation load and diffusion, therefore, became the rate determining step in establishing the efficacy of the system. Testing was conducted by the fraction negative method in which replicates of 10 spore strips were subjected to sterility testing and the fraction negative samples plotted against diffusion time. The results of these tests, which are presented in Figure 1, demonstrate that Bacillus stearothermophilus was the most resistant of the spores tested.

Based on the results of these tests, B. stearothermophilus spores on paper strips in the diffusion restricted test configuration described above was selected as the biological challenge for the validation of the STERRAD 100 Sterilization System. The STERRAD 100 Sterilization Process was validated with both the fraction negative and survivor curve test methods.

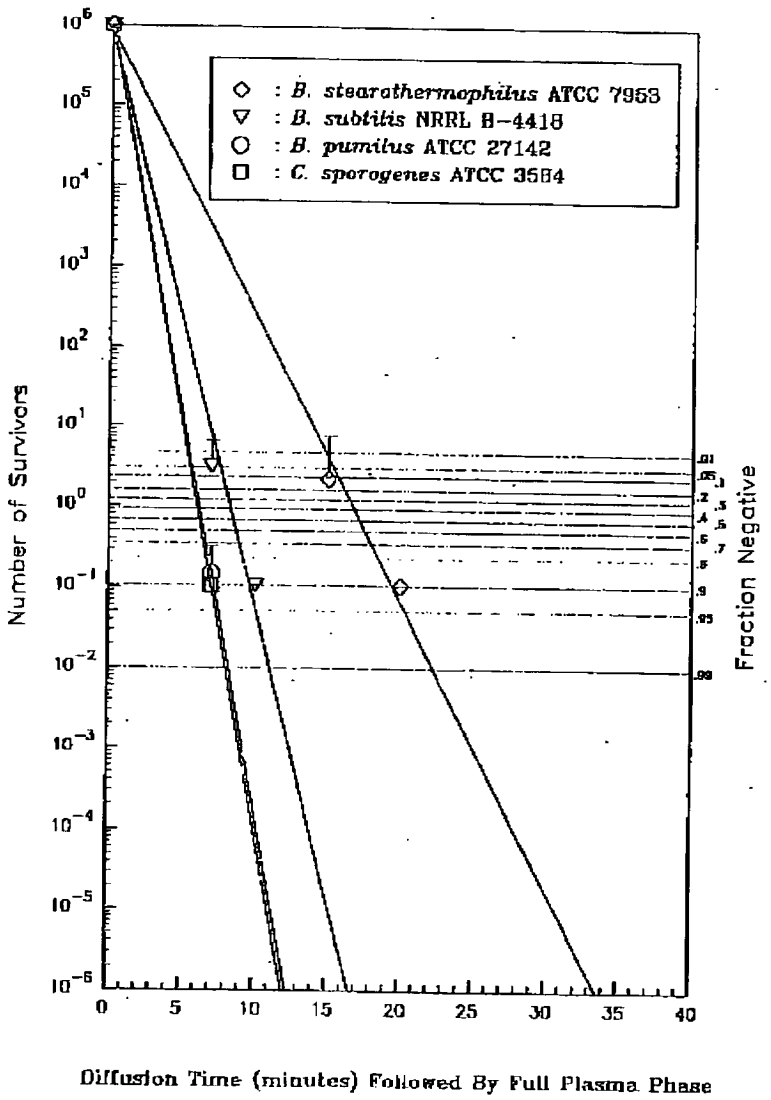

Figure 1. Kill Curves by Sterility Method (Fraction Negative) for *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus pumilus*, and *Clostridium sporogenes* Spores on Paper in Tubular Assemblies in The Validation Load.

Validation by Survivor Curve Test Method. The Survivor Curve Test Method involved the determination, by the direct plate count technique, of the number of viable B. stearothermophilus spores recovered from paper spore strips after exposure to STERRAD Sterilization Cycles with increasing diffusion times. Figure 2 contains the composite results of three sets of plate count data. Each set of data contained 10 replicates at each time point so that each point on the graph represents a total of 30 replicates. The range of variation around the points is indicated by the vertical line through the points. The linear regression line extrapolates to the 10^{-6} survivor level, on the horizontal axis at less than 30 minutes of diffusion time. The D-value calculated from the slope of the linear regression line was 1.96 minutes.

It should be noted that the linear regression line does not intersect the vertical axis at the $1x10^6$ point, the minimum initial population of spores on the paper strip. If a smooth curve was fitted to the observed averages, beginning with the starting population, a curve with an initial shoulder and increasing slope to a maximum rate of kill between 12 and 16 minutes of diffusion would be seen. This shape of kill curve is caused by the spore strips being located in a diffusion constrained load. Hydrogen peroxide must diffuse down a narrow tube to reach the spore strip. Some time in the diffusion phase of the cycle is required before the maximum achievable kill rate at the end of the plasma phase is observed. Because the eight minute survivor points are obviously still on the shoulder of the kill curve, the regression line calculated from all the 8, 12, and 16 minute data is a more conservative estimate of time to reach 10^{-6} survivors than would be the ca using only a regression line from the 12 and 16 minute time points. In spite of t conservative analysis, the intersection of the regression line with the 10^{-6} surviv line provides an SAL of 10^{-6} in less than 30 minutes of diffusion with the full minutes of plasma.

Validation by Fraction Negative Test Method. Tests were conducted by t Fraction Negative Method as described above utilizing 10 replicates of pap spore strips containing a minimum of $1x10^6$ B. stearothermophilus spores each test set. All tests were done in triplicate. As seen in Figure 3, extrapolation of the linear regression line, through the data points to the 1 survivor level, demonstrates that an SAL of 10^{-6} is achieved with less than minutes of diffusion followed by 15 minutes of plasma. The D-Value calculat from the shape of the linear regression line was 2.79 minutes. The Fracti Negative Method provides a slightly more conservative method of determining 10^{-6} SAL since the data are extrapolated from the initial spore population of 1x1 and the actual curvature of the kill curve, due to testing in a diffusion restrict location, is not considered.

The kill curve for the STERRAD Process over the entire range of test conditio is best defined by combining the results of the survivor and fraction negative t data as presented in Figure 4. The linear regression line generated using both s of data extrapolates to a 10^{-6} survivor end point that is in very good agreemen

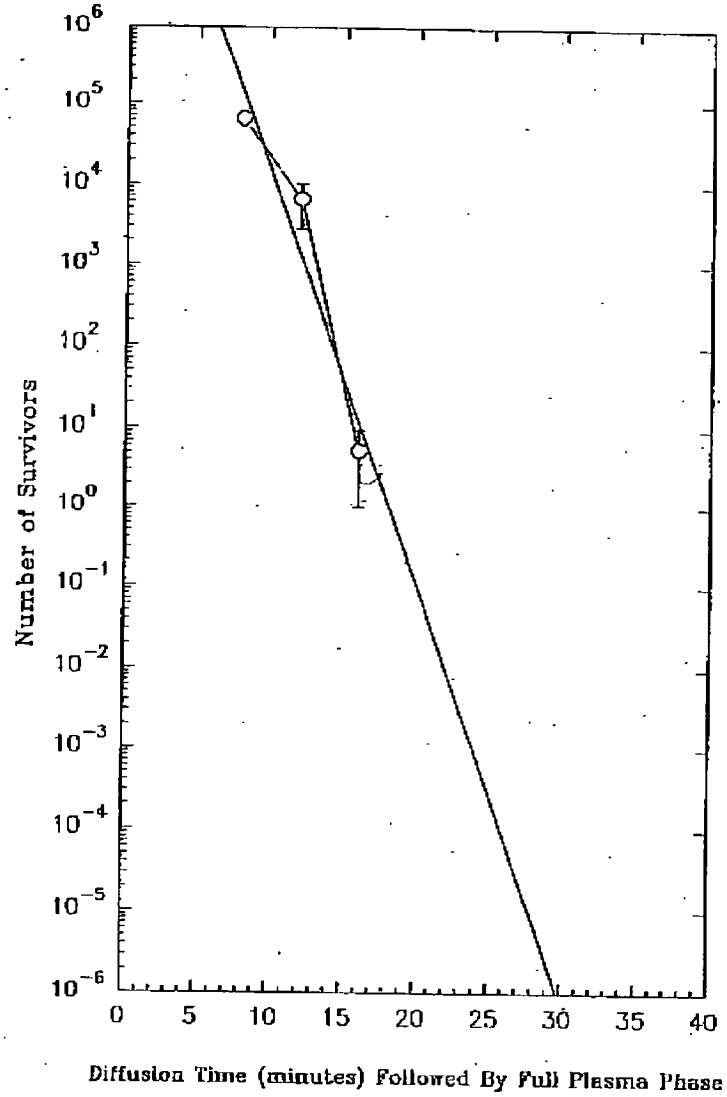

Figure 2. Composite Kill Curve by Plate Count Method for *Bacillus stearothermophilus* Spores on Paper in Tubular Assemblies in The Validation Load.

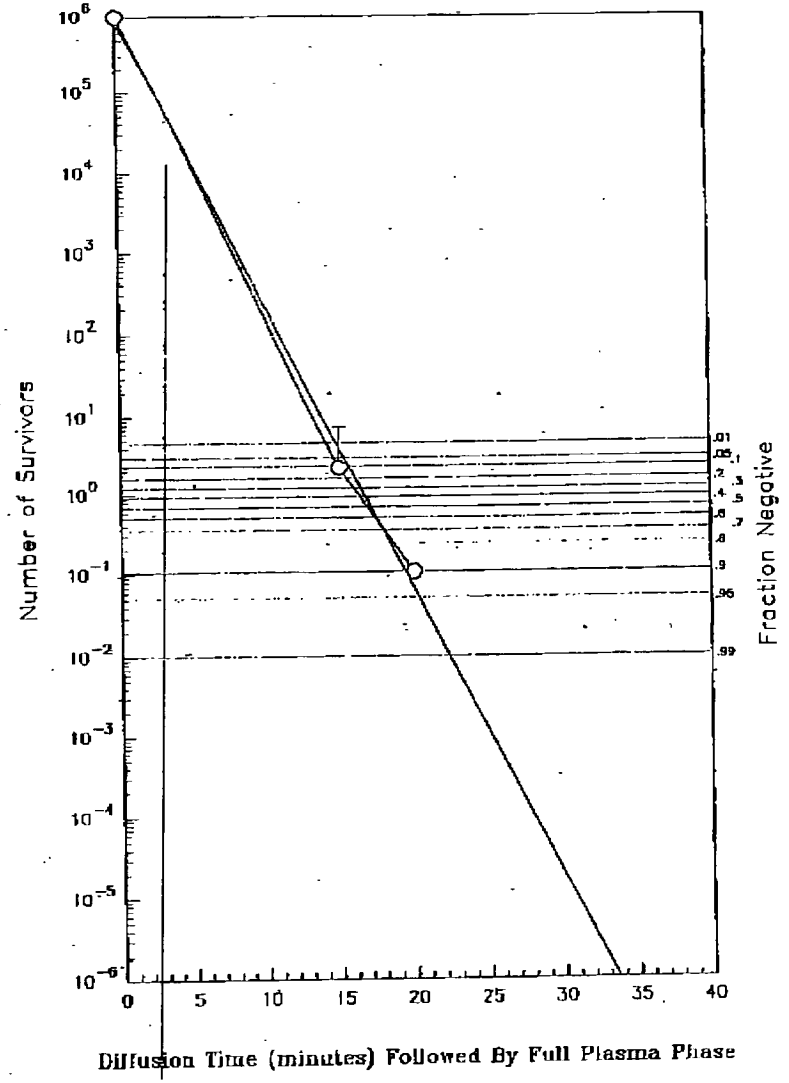

Figure 3. Composite Kill Curves by Sterility Method (Fraction Negative) for *Bacillus stearothermophilus* Spores on Paper in Tubular Assemblies in The Validation Load.

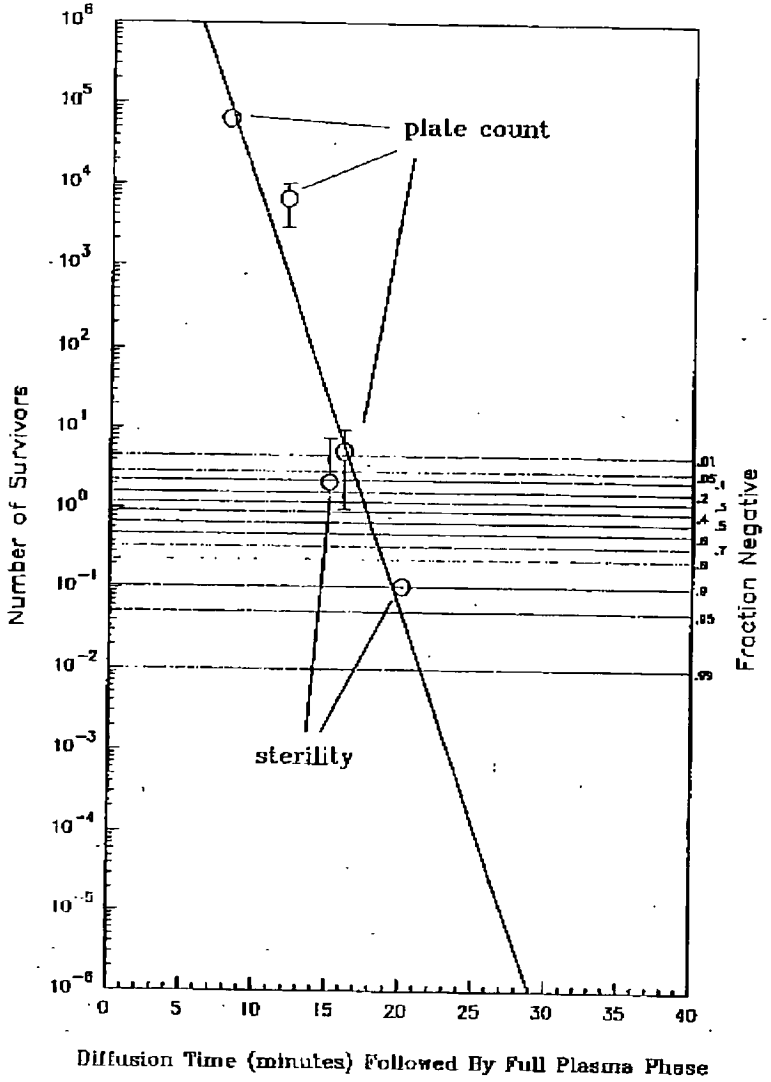

Figure 4. Composite Kill Curves by Plate Count and Sterility Methods (Fraction Negative) for *Bacillus stearothermophilus* Spores on Paper in Tubular Assemblies in The Validation Load.

with the end point obtained by the two independent test methods. This confirms that the two universally accepted test methods recommended in the AAMI standards for the validation of sterilization processes provide comparable 10^{-6} sterility survivor end points with the STERRAD Sterilization system.

Effects of Low Temperature Gas Plasma Sterilization on Medical Materials and Devices

Some of the active species formed in the hydrogen peroxide plasma are similar to those formed when *r*-radiation is used to sterilize medical devices. For example, both hydroxyl free radicals and hydroperoxyl free radicals are formed from the interaction of *r*-radiation with water molecules in the presence of oxygen. In addition, the recombination of radicals present in *r*-radiation also produces hydrogen peroxide. Although similar reactive species are present in both processes, the effect of the two processes on the physical properties of some non-metallic devices can be dramatically different. The high energy *r*-radiation is capable of passing through non-metallic materials used in medical devices and generating secondary reactions that can detrimentally affect the bulk properties of the materials. Low temperature plasmas are known to affect only a thin layer a few atoms in depth on the surface of non-metallic materials and do not affect the bulk properties of these materials. In addition, the STERRAD Sterilization System utilizes a secondary plasma that minimizes surface modification since the item to be sterilized is not exposed to the direct or primary plasma discharge.

The effect of the low temperature gas plasma process utilized in the STERR Sterilization Process on both metallic and non-metallic materials, as well complex medical devices containing a combination of these materials, has b investigated. Whenever possible, the effect of the STERRAD Sterilizat Process on the physical, chemical, and functional properties of these materials devices have been compared to the effects observed after sterilization by ano sterilization process, i.e., gamma radiation, steam, etc.

Metallic Device. Metallic medical devices have traditionally been sterilize the hospital environment by steam or by dry heat. Although most met devices withstand the temperatures associated with these processes with mini deterioration in performance, delicate cutting edges, such as exist in microsurg instruments, can be rapidly degraded by repeated exposure to high tempera and high humidity sterilization processes (*21*).

Because of the sensitivity of microsurgical instruments to high tempera sterilization processes, the effect of the STERRAD Sterilization Process microsurgical instruments were evaluated and compared to that of st sterilization. Microsurgical scissors were exposed to a total of 50 STERR Sterilization cycles. The scissors were manipulated between sterilization cycl simulate actual use of the product and tested for cutting efficiency by manufactures specified functionality protocol after every ten sterilization cyc

A second set of scissors were similarly exposed to repeated steam sterilization cycles under the same test protocol. The microsurgical scissors exposed to 50 STERRAD Sterilization cycles show no obvious change in appearance or any change in cutting efficiency. By contrast, after exposure to 10 steam sterilization cycles the surface of the microsurgical scissors were visually duller in appearance and the scissors did not pass the manufacturers test for cutting efficiency.

Similar results have been obtained in studies conducted in Germany on microsurgical ophthalmic instruments (Draeger, J., Universitats - Krankenhaus Eppendorf, Hamburg, Germany, personal communication, 1995), (*22*). Apparently, the combination of low temperature and low humidity that exists in the low temperature plasma STERRAD Sterilization Process does not adversely affect the functional properties of the metals utilized in delicate microsurgical instruments.

Non-metal Devices. As noted earlier, there is a significant similarity between the active species generated in the low temperature hydrogen peroxide plasma sterilization process utilized in the STERRAD Process and those generated during gamma irradiation sterilization. The differences in energies associated with the generation of these species are however remarkably different. For example, the accelerated electrons present in the low temperature hydrogen peroxide gas plasma process have energies in the 1 to 10 eV as compared to the gamma radiation energies that exceed 1 MeV. The low electron energies associated with low temperature gas plasma processes limit the penetration and subsequent chemical effects to the surface of non-metallic substrates where as the high energy gamma radiation is known to affect the bulk properties of these materials. To examine the difference in the effect of these two processes on a non-metallic medical substrate, a nonwoven polypropylene based fabric (Spunguard™ Heavy Duty Sterilization Wrap) was exposed to three consecutive sterilization processes by the STERRAD system and gamma irradiation. The bulk physical properties, i.e., tensile strength and elongation, of polypropylene are known to be adversely affected by gamma irradiation sterilization. The nonwoven fabric tested consists of a layer of melt blown polypropylene fibers laminated between two layers of spunbonded polypropylene fibers. The fiber diameter of the spunbonded layer is approximately 10 micron which the melt blown layer has a fiber diameter of 1 micron. The fabric therefore contains a polymer that is degraded by gamma irradiation and also has a large surface area that can chemically react with the active species generated in the low temperature gas plasma sterilization process.

Table II contains the grab tensile and elongation values for the non-woven fabric after exposure to 0 to 3 sterilization cycles with the STERRAD Sterilization Process and gamma irradiation. The results with the gamma irradiated samples are as expected. As the exposure to gamma radiation increased, i.e., from 1, 2 and 3 sterilization cycles at a dose of 2.8 Mrad per cycle, the tensile strength and elongation of the fabric decreased. The bulk properties of the polypropylene fiber are being altered by the chemical reactions occurring in the polymer. The results

with the STERRAD Sterilized samples are interesting due to the lack of effect of the process on the bulk physical properties of the polypropylene substrate. All values fell within the standard deviation for the individual test data. Low temperature gas plasma is known to be a surface phenomena and only a few angstroms on the surface of even non-metallic substrates are affected by the active species generated during the plasma phase. The alteration of a few angstroms of material at the surface of the substrate has an insignificant effect on the bulk physical properties of the material being sterilized even when the diameter of the substrate is in the 1 to 10 μ range. For this reason, low temperature gas plasma sterilization can provide some unique advantages for sterilization of non-metallic medical devices.

Table II

PHYSICAL PROPERTIES[1] OF NON-WOVEN POLYPROPYLENE FABRIC EXPOSED TO STERRAD AND GAMMA IRRADIATION STERILIZATION

	STERRAD[2]		γ-RADIATION[3]	
Cycles (#)	Tensile Strength (lb.)	Elongation (%)	Tensile Strength (lb.)	Elongation (%)
0	22.5 ± 4.1	50.5 ± 6.7	22.5 ± 4.1	50.0 ± 6.7
1	21.9 ± 4.4	50.2 ± 9.6	13.7 ± 3.1	29.8 ± 4.8
2	20.2 ± 3.9	45.8 ± 11.2	8.9 ± 2.1	19.5 ± 3.3
3	20.3 ± 3.9	45.8 ± 11.2	4.8 ± 1.3	10.3 ± 2.6

1. All physical property measurements were an average for 12 samples.
2. STERRAD Process consisted of 50 minutes of pretreatment with 6.0 mg/liter H_2O_2 followed by 15 minutes of plasma with 400 watts of power.
3. Gamma irradiation dosage was 2.8 Mrad.

Surface Effects of Low Temperature Plasma. The effect of the active spec present in low temperature gas plasma on the surface of non-metallic substrates mainly manifested by a change in the wetting properties or contact angle obser when solutions come in contact with the substrate surface. This effect is due the modification of the monolayer of molecules that reside at the substrate surfa and their interaction with the contacting solution. Since the hydrogen perox plasma, utilized in the STERRAD Process, is oxidative in nature, the tendency for hydrophobic surfaces to become more hydrophilic, i.e., contact angles w aqueous solution will decrease, after exposure to STERRAD Sterilization. T change in wetting properties is dependent on many factors, including the stren of the chemical bonds in the molecules at the surface and the hydropho properties of the material. For example Teflon, which is highly hydrophobic a has strong chemical bonds (C-F bond is about 110 Kcal/mole), is less affec than polypropylene, which is less hydrophobic and has lower energy bonds (C bond is about 90 Kcal/mole). It should also be noted that the change in cont angle observed after plasma processing is normally transient in nature and

contact angle changes with time in the direction of the original contact angle. For example, the contact angle of Teflon after exposure to the STERRAD Sterilization process changed from 98° to 89°, but within 2 days had reverted to 95°. This change in contact angle with time is reported to be due to reorientation of the molecules at the surface such that the original surface chemistry is restored (*23, 24*). This phenomena further illustrates that only a thin layer of molecules at the surface are modified by low temperature gas plasma sterilization.

Bulk Property Effects. Since plasma effects are mainly limited to the surface of non-metallic substrates, the effect on the bulk properties of these substrates are more related to the chemistry of the pretreatment or diffusion stage. In the case of the STERRAD Sterilization Process, the pretreatment stage involves the use of hydrogen peroxide in the vapor or gas phase. The potential interaction of hydrogen peroxide with the chemical structures involved in the non-metallic substrate is therefore of primary interest.

Hydrophobic non-metallic substrates, such as polyethylene, polypropylene, Teflon, etc., that have low polarity and are not capable of forming hydrogen bonds with hydrogen peroxide have excellent material compatibility with the STERRAD Sterilization Process. At the other extreme are highly hydrophilic substrates, such as cellulosic based materials, i.e., paper, linen, etc., that are highly polar and that contain multiple hydroxyl groups capable of forming hydrogen bonds with hydrogen peroxide. These materials also have large surface areas that accelerate the interaction of hydrogen peroxide with the cellulosic structure. For that reason, these materials are not recommended to be used as packaging materials in the STERRAD Sterilizer due to the high affinity of the cellulosic substrate for hydrogen peroxide and the fact that they would act as a barrier to penetration of the hydrogen peroxide into the interior of packaged items during the pretreatment stage of the process. It is also not recommended that large quantities of cellulosic based products, such as cotton, wood, etc., be sterilized in the STERRAD Sterilizer since they would act as a hydrogen peroxide sink and the proper pressures would not be achieved during the pretreatment stage. Since the STERRAD Sterilizer is software controlled, the presence of an excess of these materials in the sterilizer would result in the sterilization cycle being canceled due to low pressure in diffusion. Although the use of higher concentrations of hydrogen peroxide or longer pretreatment times would overcome these problems, these approaches would require longer plasma times to remove residual hydrogen peroxide from the substrates and would detract from the advantages of the STERRAD Sterilization Process which includes low concentrations of hydrogen peroxide and short sterilization cycles. In addition, cellulosic based materials are capable of being rapidly sterilized in almost all cases by steam sterilization.

In between the highly hydrophobic and highly hydrophilic non-metallic substrates lies a wide range of chemical structures of intermediate polarity and of varying ability to interact with hydrogen peroxide. These include silicones, polyvinyl chloride, polyvinylidene fluoride, polycarbonates, latex rubber, polyether imide,

polystyrene, polysulfone, polyamides, polyurethanes, polyacetal, etc. In general, those compounds that are capable of forming hydrogen bonds with hydrogen peroxide, i.e., polyamides, polyacetal, etc., absorb more hydrogen peroxide than those materials that do not form hydrogen bonds. In addition, those compounds that absorb more hydrogen peroxide have the potential to be more affected by exposure to repeated sterilization processing than those materials that do not absorb hydrogen peroxide. However, since there are hundreds of different chemical formulations possible with a given class of compounds, i.e., polyamides, polyurethanes, silicones, etc. It is difficult to generalize the interaction of hydrogen peroxide with a specific class of compounds.

In general, the STERRAD Sterilization Process exhibits good material compatibility with non-metallic substrates and interactions with a specific polymeric structure or the effect of repeated exposure to the sterilization process on functional properties of an individual device would need to be determined on an individual basis.

Functionality of Medical Products. Ultimately, the utility of a sterilization process is determined by its ability to repeatedly sterilize complex medical devices containing mechanical, optical, or electrical components without affecting the functional properties of these devices. Laboratory tests were conducted on specific medical devices to quantify the effect of exposure to repeated STERRAD Sterilization cycles on the functional properties of the devices. The devices selected for testing represent a wide range of materials including metals, plastics, rubber and optical surfaces which must retain properties such as flexibility, optical clarity, electrical discharge, etc., after being repeated sterilized. In all tests, the devices were manipulated between sterilization cycles to simulate actual use of the product. Devices were cycled for a total of 50 cycles and were evaluated after 10, 20, 30, and 40 cycles as well as after the 50 cycles. The total of 50 cycles was chosen for devices which normally undergo repetitive sterilization and use. If no effect is seen in 50 cycles, it was considered unlikely that any adverse effect would occur as a result of additional exposures. Functionality tests were conducted according to the device manufacturer's protocol, or by a quantifiable test procedure developed specifically for that devices. The results of tests on three medical devices a resectoscope, defibrillator paddles and a flexible fiberoptic sigmoidoscope, are presented below.

Table III contains the results of testing the electrical properties of a defibrillator set after repetitive sterilization cycles compared to a control set that was manipulated to simulate actual use of the device but was not exposed to the STERRAD Sterilization Process. As noted in the footnote, the manufacturers specification indicates that the charge and discharge values should not vary by more than 15%. The data presented in Table III illustrates that there is not a significant difference in the charge and discharge values obtained with the control and test defibrillator sets, and that both sets of data are well within the manufacturers specified limits.

Table III

EFFECT OF MULTIPLE STERRAD STERILIZATION CYCLES ON THE ELECTRICAL FUNCTIONALITY OF A DEFIBRILLATOR SET

Number of Sterilization Cycles	Electrical Test Results[1] STERRAD Treated Charge (Joules)	Discharge (Joules)	Difference (Joules)	Manipulation Control Charge (Joules)	Discharge (Joules)	Difference (Joules)
0	20.0	19.7	-0.3	20.0	19.5	-0.5
	50.0	49.9	-0.1	50.0	49.9	-0.1
10	20.0	19.7	-0.3	20.0	19.8	-0.2
	50.0	49.0	-1.0	50.0	50.3	+0.3
20	20.0	20.8	+0.8	20.0	19.6	-0.4
	50.0	49.8	-0.2	50.0	49.2	-0.8
30	20.0	19.7	-0.3	20.0	19.8	-0.2
	50.0	49.9	-0.1	50.0	50.2	+0.2
40	20.0	19.7	-0.3	20.0	19.8	-0.2
	50.0	50.4	+0.4	50.0	50.6	+0.6
50	20.0	20.2	+0.2	20.0	19.8	-0.2
	50.0	50.3	+0.3	50.0	50.2	+0.2

1 The manufacturer specifies that the difference between the charge and discharge values not exceed 15%, i.e., for a 20 Joules charge ± 3 Joules, and a 50 Joules charge ± 7.5 Joules.

Table IV shows the results of testing the electrical properties of a resectoscope versus a control. The electrical performance of the test and control devices was determined to be the best objective criterion for evaluation of this device. Analysis of the data showed that both devices were within the 15 percent deemed to be acceptable variability between the power supply alone and the power supply with working elements and cutting loop.

Table V contains the results of testing mechanical and optical properties of a flexible fiberoptic sigmoidoscope after repetitive sterilizing cycles in the STERRAD Sterilizer. The testing was done using a control endoscope for comparison. No change in either mechanical or optical properties could be observed in the test sigmoidoscope as compared to the control device.

Report from clinical test sites, as well as information obtained from hospitals who have used the STERRAD Sterilizer to repetitive sterilize medical devices for over one year, have substantiated that the STERRAD Sterilization process is compatible with a wide range of medical substrates and devices normally used in the hospital environment.

Table IV

EFFECT OF MULTIPLE STERRAD STERILIZATION CYCLES ON THE ELECTRICAL PROPERTIES OF A RESECTOSCOPE

Number of sterilization cycles	Power Setting	STERRAD Treated Power Supply Alone (watts)	Power Supply with Working Elements and Cutting Loop (watts)	Difference between Power Supply and Cutting Loop[1] (%)	Manipulation Control Power Supply Alone (watts)	Power Supply with Working Elements and Cutting Loop (watts)	Difference between Power Supply and Cutting Loop (%)
0	35	87	87	0	96	92	-4.2
	55	255	255	0	255	247	-3.1
	65	380	370	-2.6	350	360	+2.9
10	35	96	96	0	110	110	0
	55	262	262	0	275	280	+1.8
	65	380	390	+2.6	390	395	+1.3
20	35	101	92	-8.9	96	96	0
	55	270	255	-5.6	275	280	+1.8
	65	390	360	-7.7	380	390	+2.6
30	35	101	92	-8.9	101	101	0
	55	270	255	-5.6	270	280	+3.7
	65	390	380	-2.6	380	395	+3.9
40	35	87	96	+10.3	92	101	+9.8
	55	247	270	+9.3	255	270	+5.9
	65	350	380	+8.6	390	380	-2.6
50	35	96	96	0	96	106	+10.4
	55	262	262	0	270	270	0
	65	370	370	0	380	380	0

1 The specification for this test, which was developed in-house, states that the difference between the power supply alone and the power supply and cutting loop exceed 15%.

Table V

EFFECT OF MULTIPLE STERRAD STERILIZATION CYCLES ON THE OPTICAL AND MECHANICAL PROPERTIES OF A FIBEROPTIC SIGMOIDOSCOPE

Number of Sterilization Cycle	STERRAD Treated Optical Properties	Angulation Control	Manipulation Control Optical Properties	Angulation Control
0	Unchanged	Unchanged	Unchanged	Unchanged
10	Unchanged	Unchanged	Unchanged	Unchanged
20	Unchanged	Unchanged	Unchanged	Unchanged
30	Unchanged	Unchanged	Unchanged	Unchanged
40	Unchanged	Unchanged	Unchanged	Unchanged
50	Unchanged	Unchanged	Unchanged	Unchanged

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